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(54) Title: NEW POLYNUCLEOTIDES AND POLYPEPTIDES OF THE HGH-V GENE

(57) Abstract: The present invention relates to new polynucleotides deriving from the nucleotide sequence of the hGH-V gene and comprising SNP(s), new polypeptides comprising a mutation caused by this (these) SNP(s) as well as their therapeutic uses.

NEW POLYNUCLEOTIDES AND POLYPEPTIDES OF THE hGH-V GENE

RELATED APPLICATIONS:

The present invention claims priority to:

- US provisional application 60/296149 filed on June 07, 2001 titled "Nouveaux polynucléotides et polypeptides de l'hormone de croissance humaine"
- European application 01402435.0 filed on September 21, 2001 titled "New polynucleotides and polypeptides of the hGH-V gene"
- US provisional application 60/325,401 filed on September 27, 2001 titled "New polynucleotides and polypeptides of the hGH-V gene".

BACKGROUND OF THE INVENTION

Field of the Invention.

The present invention relates to new polynucleotides deriving from the nucleotide sequence of the hGH-V gene and comprising new SNP(s), new polypeptides comprising a mutation caused by this (these) SNP(s), as well as their therapeutic uses.

Related Art

The human placental growth hormone gene, hereinafter referred to as hGH-V, is described in the publications :

- George,D.L., Phillips,J.A. III, Francke,U. and Seeburg,P.H. (1981). The genes for growth hormone and chorionic somatomammotropin are on the long arm of human chromosome 17 in region q21 to qter. Hum. Genet. 57 (2), 138-141.
- Harper,M.E., Barrera-Saldana,H.A. and Saunders,G.F. (1982). Chromosomal localization of the human placental lactogen-growth hormone gene cluster to 17q22-24. Am. J. Hum. Genet. 34 (2), 227-234.
- Kidd,V.J. and Saunders,G.F. (1982). Linkage arrangement of human placental lactogen and growth hormone genes. J. Biol. Chem. 257 (18), 10673-10680.
- Barsh,G.S., Seeburg,P.H. and Gelinas,R.E. (1983). The human growth hormone gene family: structure and evolution of the chromosomal locus. Nucleic Acids Res. 11 (12), 3939-3958.

- Igout,A., Scippo,M.L., Frankenne,F. and Hennen,G. (1988). Cloning and nucleotide sequence of placental hGH-V cDNA. Arch. Int. Physiol. Biochim. 96 (1), 63-67.

- Cooke,N.E., Ray,J., Emery,J.G. and Liebhaber,S.A. (1988). Two distinct species of human growth hormone-variant mRNA in the human placenta predict the expression of novel growth hormone proteins. J. Biol. Chem. 263 (18), 9001-9006.

- Chen,E.Y., Liao,Y.C., Smith,D.H., Barrera-Saldana,H.A., Gelinas,R.E. and Seeburg,P.H. The human growth hormone locus. Genomics 4 (4), 479-497.

- Vnencak-Jones,C.L. and Phillips,J.A. III. (1990). Hot spots for growth hormone gene deletions in homologous regions outside of Alu repeats. Science 250 (4988), 1745-1748.

- MacLeod,J.N., Lee,A.K., Liebhaber,S.A. and Cooke,N.E. (1992). Developmental control and alternative splicing of the placentally expressed transcripts from the human growth hormone gene cluster. J. Biol. Chem. 267 (20), 14219-14226.

- Boguszewski,C.L., Svensson,P.A., Jansson,T., Clark,R., Carlsson,L.M. and Carlsson,B. (1998). Cloning of two novel growth hormone transcripts expressed in human placenta. J. Clin. Endocrinol. Metab. 83 (8), 2878-2885.

The nucleotide sequence of this gene is accessible under accession number JO3071 in the GenBank database.

Placental growth hormone (PGH) is the product of the hGH-V gene.

The human growth hormone (hGH)/human placental lactogen (hPL) gene family, which consists of two hGH (pituitary GH, also called adult GH, and placental GH) and three hPL genes, is involved in the regulation of maternal and fetal metabolism and the growth and development of the fetus.

During pregnancy, pituitary GH (hGH-N) expression in the mother is suppressed and hGH-V, that is a GH variant expressed by the cells from the syncytiotrophoblast layer of the human placenta, becomes the predominant GH in the mother. Assays of PGH by specific monoclonal antibodies reveal that, in the maternal circulation from 15-20 weeks up to term, PGH gradually replaces pituitary growth hormone which becomes undetectable (Alsat et al. (1998); Physiological role of human placental growth hormone. Mol. Cell Endocrinol. 140 :121-127).

hGH-N, which is expressed by the fetal pituitary, has little or no physiological actions in the fetus until late in pregnancy due to the lack of functional GH receptors on fetal tissues (Handwerger et al. (2000); The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development; J. Pediatr Endocrinol. Metab. 13:343-356).

By contrast, PGH is continuously secreted by the placenta.

Similarly to pituitary growth hormone, placental growth hormone (in particular its main isoform NO. 1 as defined below) has high somatogenic and low lactogenic activities. PGH secretion appears to have important implications for physiological adjustment to gestation and especially in the control of maternal IGF-1 levels. Thus, it acts as a growth-promoting hormone and appears to be the main stimulator of insulin-like growth factor I (IGF-1) secretion (Caron et al. (1997); Expression of somatostatin receptor SST4 in human placenta and absence of octreotide effect on human placental growth hormone concentration during pregnancy. J. Clin. Endocrinol. Metab. 82:3771-3776).

Moreover, PGH may continue to have an effect on the small child after birth, as suggested by the Karlberg's model, which corresponds to a model differentiating between three discrete, but related periods of postnatal growth : fetal/infant, childhood and pubertal phases (Karlberg J. (1990); The infancy-childhood growth spurt. Acta. Paediatr. Scand. Suppl. 367:111-118). During these periods, growth is regulated by different hormonal control systems. In particular, the infant period is commonly seen as a continuation of fetal life and the insulin like growth factor system is proposed to be the most important factor regulating growth. Growth in childhood is affected by both environmental and genetic influences.

It has been demonstrated that PGH may be used as a predictive factor for fetal aneuploidy and pregnancy rhinitis (Moghadam *et al.* (1998) Fetal Diagnosis and therapy 13:291-297 ; Ellegard *et al.* (1998) Archives of Otolaryngology and Head surgery 124: 439-443).

PGH may not only act on fetal or young child growth. Indeed, it has been shown by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis that hGH-N and hGH-V transcripts are simultaneously produced by human peripheral blood

mononuclear cells in both men and women as well as pregnant women. Thus, hGH-V mRNA is expressed by cells other than the syncytiotrophoblast, it is not regulated by PIT-1 (pituitary transcription factor 1), and may be involved in immune regulation, as is pituitary GH (Melen et al. (1997). Both pituitary and placental growth hormone transcripts are expressed in human peripheral blood mononuclear cells (PBMC) (Clin. Exp. Immunol. 110:336-340).

The biological actions of placental growth hormone are mediated through binding to specific, high affinity receptors localized to the plasma membrane of target tissues. The affinity of placental growth hormone for binding to the GH receptor is identical to that of pituitary growth hormone. The expression of GH receptor mRNA in human fetal tissues has been examined using the technique of reverse transcription-polymerase chain reaction (RT-PCR). Messenger RNA encoding the membrane-bound GH receptor is expressed in the liver, kidney, skin, muscle, lung, adrenal, brain, spleen, intestine and pancreas of the human fetus at 7-20 weeks of gestation. Immunohistochemistry analysis indicates a progressive maturation of GH receptor in late gestation or in the perinatal period (Handwerger and Freemark (2000); The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. J. Pediatr. Endocrinol. Metab. 13:343-356). As a consequence, growth hormone may exert growth-promoting and metabolic effects on many different tissues.

Information concerning the physiological role of human placental growth hormone can be found in additional articles and reviews like :

- Alsat et al. (1997). Human placental growth hormone. Am. J. Obstet. Gynecol. 177:1526-1534.
- Chappel and Murphy (2000). Growth hormone. In : Cytokine reference, Academic Press. 251-265.

Human growth hormone modulates the production of IGF-1 in the pregnant mother, and plays important roles in perinatal carbohydrate metabolism, phallic growth and craniofacial developments.

The physiological activity for which human pituitary GH is best known is the promotion of growth of bone, cartilage and soft tissue. Pituitary GH appears also to

control important immune functions. GH has been shown to be produced by T-cells, B-cells and macrophages. Receptors for GH releasing hormone have also been found on cells of the immune system. GH appears to act as an enhancer of immune responses and is produced in considerable amounts by T-helper cells.

Hypothalamus-derived GH releasing hormone has been shown recently to elicit GH production by lymphocytes. GH augments the cytolytic activity of T-cells, antibody synthesis, and granulocyte differentiation induced by GM-CSF. GH also enhances production of TNF-alpha, generation of superoxide anions from peritoneal macrophages, and natural killer activity. GH induces a chemotactic response in human monocytes which is inhibited by somatostatin. GH enhances the synthesis of some thymic hormones. An active fragment of GH appears to account for some of the biological activity of PM (pregnancy mitogen). GH has been shown to promote engraftment of murine or human T-cells in severe combined immunodeficient mice (SCID).

Treatment of mice with recombinant human GH has been shown to partially counteract the myelosuppressive properties of azidothymidine, resulting in an increase in splenic hematopoietic progenitor cells. GH has been shown to function as a paracrine mediator of growth and differentiation in the hematopoietic system.

A recently reported action of GH is its support of angiogenesis.

Many recent studies on adults lacking growth hormone demonstrated that growth hormone exerts an effect on lipid metabolism and prevents arteriosclerosis.

PGH secretion has important implications in the control of maternal IGF-1 levels.

Deficiency of IGF-1 was proposed as the nature of the basic defect in the African pygmy and possibly also in the Laron type of dwarfism.

IGF-1 gene may be the site of the mutation causing one form of hypochondroplasia.

IGF-1 stimulates skeletal muscle hypertrophy and a switch to glycolytic metabolism by activating the calcium calmodulin-dependent phosphatase calcineurin and inducing the nuclear translocation of transcription factor NFATC1.

A strong association between circulating IGF-1 concentrations and the risk of breast cancer in premenopausal women has been demonstrated. Holly discussed the evidence that high levels of circulating IGF-1 pose a risk of breast cancer in premenopausal women, and noted that a similar association has been reported for prostate cancer (Holly (1998). Insulin-like growth factor-I and new opportunities for cancer prevention. Lancet 351: 1373-1375).

Circulating IGF-1 may play a role in the age-related reduction of certain cognitive functions, specifically speed of information processing (Aleman et al. (1999). Insulin-like growth factor-I and cognitive function in healthy older men. J. Clin. Endocr. Metab. 84: 471-475).

IGF-1 may contribute to transformation, cell migration, and a propensity for metastasis *in vivo* (Playford et al. (2000). Insulin-like growth factor 1 regulates the location, stability, and transcriptional activity of beta-catenin. Proc. Nat. Acad. Sci. 97: 12103-12108).

A 15-year-old boy with severe prenatal and postnatal growth failure, sensorineural deafness, and mental retardation who was homozygous for a partial deletion of the IGF-1 gene has been described (Woods et al. (1996). Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. New Eng. J. Med. 335: 1363-1367).

Rasmussen *et al.* considered the IGF-1 and IGF-1R genes as candidates for low birth weight, insulin resistance, and type II diabete (Rasmussen et al. (2000). Studies of the variability of the genes encoding the insulin-like growth factor I receptor and its ligand in relation to type 2 diabetes mellitus. J. Clin. Endocr. Metab. 85: 1606-1610).

As indicated by experiments on knock-out mouse, IGF-1 may have specific roles in axonal growth and myelination. In addition, neonatal mortality is substantial, suggesting that the defect may be lethal in humans also.

IGF, which is probably produced also by stromal cells of the bone marrow also influences hematopoiesis, in particular the development of the red blood cell lineage. In serum-free cultures IGF-1 functions as a colony stimulating factor for erythroid cells.

Hellstrom et al. showed that lack of IGF-1 in knockout mice prevents normal retinal vascular growth, despite the presence of vascular endothelial growth factor,

which is important for vessel development (Hellstrom et al. (2001). Low IGF-1 suppresses VEGF-survival signaling in retinal endothelial cells: direct correlation with clinical retinopathy of prematurity. Proc. Nat. Acad. Sci. 98: 5804-5808).

The nucleotide sequence of the wild-type hGH-V gene of reference, mentioned in the GenBank accession number JO3071, comprises 5001 nucleotides.

However, it appears that this nucleotide sequence is not exact. The inventor has noted that the nucleotide coding sequence of the isoform NO. 2 of the hGH-V gene reported in the GenBank database (accession number: NM_022557) comprises an additional cytidine (c) at position 480. This nucleotide coding sequence encodes for the amino acid sequence of PGH isoform NO. 2 as shown by SwissProt database NO. P09587.

By contrast, the nucleotide sequence of hGH-V gene deposited in the Genbank database (accession number: J03071) as such does not comprise a cytidine (c) between position 3194 and 3195. Such a nucleotide sequence would be responsible for an altered PGH isoform NO. 2 due to the apparition of a frameshift, which would be different from PGH isoform NO. 2 deposited in the SwissProt database NO. P09587.

Consequently, to be in agreement with the coding sequence of the isoform NO. 2 of the hGH-V gene reported in the GenBank database (accession number: NM_022557), the inventor has considered that a correction was necessary. This correction consists in adding a cytidine (c) between positions 3194 and 3195 in the nucleotide sequence of hGH-V gene deposited in the GenBank database (accession number: J03071). This corrected sequence is named hereinafter "corrected nucleotide sequence of wild-type hGH-V gene".

The hGH-V primary transcript undergoes alternative splicing pathways generating at least four different messenger RNAs, predicting the expression of four different PGH isoforms. The four PGH isoforms will be called isoform NO. 1, NO. 2, NO. 3 and NO. 4.

The predominant PGH isoform, hereinafter referred to as isoform NO. 1, is encoded by a coding nucleotide sequence composed of 654 nucleotides, which utilizes all five exons of the corrected nucleotide sequence of the wild-type hGH-V gene, which have the following positions in the nucleotide sequence SEQ ID NO. 1:

- Exon 1: nucleotide 2143 to 2152;
- Exon 2: nucleotide 2425 to 2585;
- Exon 3: nucleotide 2796 to 2915;
- Exon 4: nucleotide 3007 to 3171; and
- Exon 5: nucleotide 3425 to 3622.

This PGH isoform encoded by hGH-V is composed of 217 amino acids and corresponds to a protein of 22-kDa. The 217 amino acid sequence corresponds to an immature protein, that will be converted to a mature protein of 191 amino acids, by cleavage of the signal peptide that includes the first 26 amino acids. The nucleotide coding sequence of the isoform NO. 1 is accessible under accession number NM_002059 in the GenBank database.

Isoform NO. 2 utilizes an intron situated between exons 4 and 5 to generate the longest isoform of 256 amino acids, which diverges from all other GH isoforms in the carboxy terminus. This isoform is encoded by a coding nucleotide sequence composed of 771 nucleotides corresponding to nucleotides 2143 to 2152; nucleotides 2425 to 2585, nucleotides 2796 to 2915 and nucleotides 3007 to 3486 of the corrected nucleotide sequence of wild-type hGH-V gene. The 256 amino acid sequence corresponds to an immature protein, that will be converted to a mature protein of 230 amino acids, by cleavage of the signal peptide that includes the first 26 amino acids. The nucleotide coding sequence of the isoform NO. 2 is accessible under accession number NM_022557 in the GenBank database.

Isoform NO. 3 utilizes an alternative splice donor site in exon 4 causing a 4 nucleotide deletion and a frameshift which generates an isoform of 245 amino acids with an unique carboxy-terminus. This isoform is encoded by a coding nucleotide sequence composed of 738 nucleotides corresponding to nucleotides 2143 to 2152; nucleotides 2425 to 2585, nucleotides 2796 to 2915, nucleotides 3007 to 3167 and nucleotide 3425 to 3710 of the corrected nucleotide sequence of wild-type hGH-V gene. The 245 amino acid sequence corresponds to an immature protein, that will be converted to a mature protein of 219 amino acids, by cleavage of the signal peptide that includes the first 26 amino acids. The nucleotide coding sequence of the isoform NO. 3 is accessible under accession number NM_022558 in the GenBank database.

Isoform NO. 4 utilizes an alternative splice acceptor site of 45 nucleotides into exon 3 to generate the 20-kDa isoform composed of 202 amino acids, which has an internal deletion relative to the predominant 22-kDa isoform (isoform NO. 1). This isoform is encoded by a coding nucleotide sequence composed of 609 nucleotides corresponding to nucleotides 2143 to 2152; nucleotides 2425 to 2585, nucleotides 2841 to 2915, nucleotides 3007 to 3171 and nucleotide 3425 to 3622 of the corrected nucleotide sequence of the wild-type hGH-V gene. The 202 amino acid sequence corresponds to an immature protein, that will be converted to a mature protein of 176 amino acids, by cleavage of the signal peptide that includes the first 26 amino acids. The nucleotide coding sequence of the isoform NO. 4 is accessible under accession number NM_022556 in the GenBank database.

The PGH is involved in different human disorders and/or diseases, such as:

- disorders or diseases linked to the human growth and development, such as fetal growth and development, perinatal carbohydrate metabolism, phallic growth, craniofacial developments, hypochondroplasia, Laron type of dwarfism,
- disorders related to IGF-1 secretion, such as cognitive functions reduction, mental retardation, sensorineural deafness, insulin resistance, type II diabete, hematological disorders,
- tumors and cancers such as breast cancer and prostate cancer,
- disorders or diseases linked to the immune system such as allergies, auto-immune diseases, graft rejection, and certain infectious diseases,
- metabolic disorders or diseases related to lipid, nitrogen and/or carbohydrate metabolism such as obesity, arteriosclerosis, body mass maintenance,
- disorders or diseases linked to angiogenesis, retinopathy, cardiovascular diseases.

Nevertheless, growth hormone may have numerous side effects when it is used in pharmaceutical compositions, such as evolutive cancers, formation of antibodies directed against growth hormone, intracranial hypertensions, insuline resistance, hypoglycemia or hyperglycemia, incompatibility with glucocorticoids, disorders in thyroid functions, vision troubles, nausea and/or vomiting, liquid retention, acromegalia syndrom, lipodistrophia and hip bone necrosis.

The inventor has found new polypeptide and new polynucleotide analogs to the hGH-V gene capable of having a different functionality from the natural wild-type placental or pituitary GH proteins.

These new polypeptides and polynucleotides can notably be used to treat or prevent the disorders or diseases previously mentioned and avoid all or part of the disadvantages, which are tied to them.

BRIEF SUMMARY OF THE INVENTION

The nucleotide sequence SEQ ID NO. 1 corresponds to the corrected nucleotide sequence of the wild-type hGH-V gene mentioned above comprising 5002 nucleotides.

The nucleotide coding sequence SEQ ID NO. 2 corresponds to the nucleotide coding sequence of the isoform NO. 1 of the hGH-V gene, it comprises 654 nucleotides.

The nucleotide coding sequence SEQ ID NO. 3 corresponds to the nucleotide coding sequence of the isoform NO. 2 of the hGH-V gene, it comprises 771 nucleotides.

The nucleotide coding sequence SEQ ID NO. 4 corresponds to the nucleotide coding sequence of the isoform NO. 3 of the hGH-V gene, it comprises 738 nucleotides.

The nucleotide coding sequence SEQ ID NO. 5 corresponds to the nucleotide coding sequence of the isoform NO. 4 of the hGH-V gene, it comprises 609 nucleotides.

The applicant has identified 15 SNPs in the corrected nucleotide sequence of the wild-type hGH-V gene.

These 15 SNPs are the following: g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c, c3693a.

It is understood, in the sense of the present invention, that the numbering corresponding to the positioning of the SNPs previously defined is relative to the numbering of the nucleotide sequence SEQ ID NO. 1. The letters a, t, c and g correspond, respectively, to the nitrogenous bases adenine, thymine, cytosine and guanine. The first letter corresponds to the wild-type nucleotide, whereas the last letter corresponds to the mutated nucleotide.

The SNP g2139c corresponds to the substitution of a nucleotide guanine (g) by a nucleotide cytosine (c) at position 2139 of the corrected nucleotide sequence of the wild-type hGH-V gene.

The SNP g2197t corresponds to the substitution of a nucleotide guanine (g) by a nucleotide thymine (t) at position 2197 of the corrected nucleotide sequence of the wild-type hGH-V gene.

The SNP g2204a corresponds to the substitution of a nucleotide guanine (g) by a nucleotide adenine (a) at position 2204 of the corrected nucleotide sequence of the wild-type hGH-V gene.

The SNP g2205t corresponds to the substitution of a nucleotide guanine (g) by a nucleotide thymine (t) at position 2205 of the corrected nucleotide sequence of the wild-type hGH-V gene.

The SNP c2206t corresponds to the substitution of a nucleotide cytosine (c) by a nucleotide thymine (t) at position 2206 of the corrected nucleotide sequence of the wild-type hGH-V gene.

The SNP g2267a corresponds to the substitution of a nucleotide guanine (g) by a nucleotide adenine (a) at position 2267 of the corrected nucleotide sequence of the wild-type hGH-V gene.

The SNP g2435a corresponds to the substitution of a nucleotide guanine (g) by an adenine (a) at position 2435 of the corrected nucleotide sequence of the wild-type hGH-V gene. This SNP is located at position 10 of the nucleotide coding sequence of isoforms NO. 1, 2, 3 and 4. This SNP is hereinafter designated as g2435a or g10a according to whether one refers to the position on the nucleotide sequence SEQ ID NO. 1 or on the nucleotide coding sequences SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, and SEQ ID NO. 5.

The SNP c2465a corresponds to the substitution of a nucleotide cytosine (c) by a nucleotide adenine (a) at position 2465 of the corrected nucleotide sequence of the wild-type hGH-V gene. This SNP is located at position 51 of the nucleotide coding sequence of isoforms NO. 1, 2, 3 and 4. This SNP is hereinafter designated as c2465a or c51a according to whether one refers to the position on the nucleotide sequence SEQ ID NO. 1 or on the nucleotide coding sequences SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, and SEQ ID NO. 5.

The SNP a2635c corresponds to the substitution of a nucleotide adenine (a) by a nucleotide cytosine (c) at position 2635 of the corrected nucleotide sequence of the wild-type hGH-V gene.

The SNP t3169g corresponds to the substitution of a nucleotide thymine (t) by a nucleotide guanine (g) at position 3169 of the corrected nucleotide sequence of the wild-type hGH-V gene. This SNP is located at position 454 of the nucleotide coding sequence of isoforms NO. 1, and 2, and at position 409 of the nucleotide coding sequence of isoform NO. 4. This SNP is hereinafter designated as t3169g by reference to its position on the nucleotide sequence SEQ ID NO. 1, or t454g by reference to its position on the nucleotide coding sequences SEQ ID NO. 2 and SEQ ID NO. 3, or t409g by reference to its position on the nucleotide coding sequence SEQ ID NO. 5.

The SNP g3267c corresponds to the substitution of a nucleotide guanine (g) by a nucleotide cytosine (c) at position 3267 of the corrected nucleotide sequence of the wild-type hGH-V gene. This SNP is located at position 552 of the nucleotide coding sequence of isoform NO. 2. This SNP is hereinafter designated as g3267c by reference to its position on the nucleotide sequence SEQ ID NO. 1 or g552c by reference to its position on the nucleotide coding sequence SEQ ID NO. 3.

The SNP g3343a corresponds to the substitution of a nucleotide guanine (g) by a nucleotide adenine (a) at position 3343 of the corrected nucleotide sequence of the wild-type hGH-V gene. This SNP is located at position 628 of the nucleotide coding sequence of isoform NO. 2. This SNP is hereinafter designated as g3343a by reference to its position on the nucleotide sequence SEQ ID NO. 1 or g628a by reference to its position on the nucleotide coding sequence SEQ ID NO. 3.

The SNP t3436c corresponds to the substitution of a nucleotide thymine (t) by a nucleotide cytosine (c) at position 3436 of the corrected nucleotide sequence of the wild-type hGH-V gene. This SNP is located at position 468 of the nucleotide coding sequence of isoform NO. 1, at position 721 of the nucleotide coding sequence of isoform NO. 2, at position 464 of the nucleotide coding sequence of isoform NO. 3, and at position 423 of the nucleotide coding sequence of isoform NO. 4. This SNP is hereinafter designated as t3436c by reference to its position on the nucleotide sequence SEQ ID NO. 1, t468c by reference to its position on the nucleotide coding sequence

SEQ ID NO. 2, t721c by reference to its position on the nucleotide coding sequence SEQ ID NO. 3, t464c by reference to its position on the nucleotide coding sequence SEQ ID NO. 4, or t423c by reference to its position on the nucleotide coding sequence SEQ ID NO. 5.

The SNP a3655c corresponds to the substitution of a nucleotide adenine (a) by a nucleotide cytosine (c) at position 3655 of the corrected nucleotide sequence of the wild-type hGH-V gene. This SNP is located at position 683 of the nucleotide coding sequence of isoform NO. 3. This SNP is hereinafter designated as a3655c by reference to its position on the nucleotide sequence SEQ ID NO. 1, or a683c by reference to its position on the nucleotide coding sequence SEQ ID NO. 4.

The SNP c3693a corresponds to the substitution of a nucleotide cytosine (c) by a nucleotide adenine (a) at position 3693 of the corrected nucleotide sequence of the wild-type hGH-V gene. This SNP is located at position 721 of the nucleotide coding sequence of isoform NO. 3. This SNP is hereinafter designated as c3693a by reference to its position on the nucleotide sequence SEQ ID NO. 1, or c721a by reference to its position on the nucleotide coding sequence SEQ ID NO. 4.

The first object of the invention concerns new polynucleotides defined as the corrected nucleotide sequence of the hGH-V gene but differing from it in that it comprises at least one of the following SNPs (Single Nucleotide Polymorphism): g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c, c3693a.

Preferably, one object of the invention concerns new polynucleotides defined as the corrected nucleotide sequence of the hGH-V gene but differing from it in that it comprises the SNP c2465a.

These SNPs have been identified by the applicant using the determination process described in applicant's patent application FR 00 22894, entitled "Process for the determination of one or several functional polymorphism(s) in the nucleotide sequence of a preselected functional candidate gene and its applications" and filed December 6, 2000, cited here by way of reference.

The process described in this patent application permits the identification of one (or several) preexisting SNPs in at least one individual from a random population of

individuals.

In the scope of the present invention, a fragment of the nucleotide sequence of the hGH-V gene, comprising, for example, the coding sequence, was isolated from different individuals in a population of individuals chosen in a random manner.

Sequencing of these fragments was then carried out on certain of these samples having a heteroduplex profile (that is a profile different from that of the reference wild-type hGH-V gene sequence) after analysis by DHPLC ("Denaturing-High Performance Liquid Chromatography").

The fragment sequenced in this way was then compared to the nucleotide sequence of the fragment of the reference wild-type hGH-V gene and the SNPs in conformity with the invention identified.

Thus, the SNPs are natural and present in certain individuals of the world population. In particular, SNP c2465a is present in the South American population.

According to the splicing sites used during the maturation of the transcripts (as described above), the reference wild-type hGH-V gene may code for four PGH isoforms. In isoform NO. 1, the immature PGH protein is made of 217 amino acids, corresponding to the amino acid sequence SEQ ID NO. 10, that will be converted to a mature protein of 191 amino acids, by cleavage of the signal peptide that includes the first 26 amino acids. In isoform NO. 2, the immature PGH protein is made of 256 amino acids, corresponding to the amino acid sequence SEQ ID NO. 11, that will be converted to a mature protein of 230 amino acids, by cleavage of the signal peptide that includes the first 26 amino acids. In isoform NO. 3, the immature PGH protein is made of 245 amino acids, corresponding to the amino acid sequence SEQ ID NO. 12, that will be converted to a mature protein of 219 amino acids, by cleavage of the signal peptide that includes the first 26 amino acids. In isoform NO. 4, the immature PGH protein is made of 202 amino acids, corresponding to the amino acid sequence SEQ ID NO. 13, that will be converted to a mature protein of 176 amino acids, by cleavage of the signal peptide that includes the first 26 amino acids.

The SNP c2465a (or c51a) causes a modification of the PGH protein isoforms NO. 1, NO. 2, NO. 3, and NO. 4. This SNP introduces a stop codon "tga" at positions 2463-2465 on the nucleotide sequence of the hGH-V gene and at positions 49-51 on the

nucleotide coding sequence of isoforms NO. 1 to NO. 4 of the hGH-V gene (SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, and SEQ ID NO. 5, respectively).

As a consequence, this SNP is responsible for the formation of a stop codon, during the translation process, at position 17 of the immature PGH protein isoforms encoded by the nucleotide coding sequences SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, and SEQ ID NO. 5. The resulting amino acid sequence corresponds to the first sixteen amino acids of the signal peptide. This SNP will also be called C17stop by reference to the amino acid sequences SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12 and SEQ ID NO. 13.

The amino acid sequence SEQ ID NO. 7 corresponds to the first sixteen amino acids of the immature PGH protein.

The nucleotide sequence SEQ ID NO. 6 corresponds to the nucleotide coding sequence, deduced from the hGH-V gene, encoding for the amino acid sequence SEQ ID NO. 7. The nucleotide coding sequence SEQ ID NO. 6 contains a nucleotide adenine (a) at position 51.

The SNP t3169g causes a modification of the PGH protein isoforms encoded by the nucleotide coding sequences SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, and SEQ ID NO. 5. This SNP introduces a glycine (G) instead of a tryptophan (W) (wild-type amino acid) at position 152 of the amino acid sequences SEQ ID NO. 10 and SEQ ID NO. 11, which correspond to the amino acid sequence of the immature PGH variant NO. 1 and immature PGH isoform NO. 2, respectively. This SNP is located on one of the splicing sites (situated at position 3168-3169 in the nucleotide sequence SEQ ID NO. 1) normally used in the formation of the wild-type isoform NO. 3. As a consequence, the mutated nucleotide cannot be part of a splicing site anymore, the protein composition will then be affected by this mutation. In particular, this SNP generates a mutated protein whose amino acid sequence only shares the first 150 amino acids of immature wild-type isoform NO. 3, the rest of the protein being different. Moreover, the analysis carried out on the nucleotide sequence of the hGH-V gene and the position of the possible splicing sites allows us to predict that the mutated splicing site, which would otherwise have generated the wild-type isoform NO. 3, may be replaced by a next one. The most probable splicing site is the one located at the vicinity

of the mutated splicing site, 4 nucleotides further in the 3' direction, since it is the normal splicing site used for the formation of the wild-type isoform NO. 1. As a consequence, the SNP t3169g will lead to a deficiency in wild-type isoform NO. 3 and to the synthesis of isoform NO. 1 having the SNP W152G. This SNP introduces a glycine (G) instead of a tryptophan (W) at position 137 of the sequence SEQ ID NO. 13, which corresponds to the amino acid sequence of the immature PGH isoform NO. 4. This SNP will also be called I151splice by reference to its effect on the isoform NO. 3 (amino acid sequence SEQ ID NO. 12), W152G by reference to the isoforms NO. 1 and NO. 2 (amino acid sequences SEQ ID NO. 10 and SEQ ID NO. 11, respectively), or W137G by reference to isoform NO. 4 (amino acid sequence SEQ ID NO. 13).

The SNP g3343a causes a modification of the PGH protein isoform encoded by the nucleotide coding sequence SEQ ID NO. 3. This SNP introduces a lysine (K) instead of a glutamic acid (E) (wild-type amino acid) at position 210 of the amino acid sequence SEQ ID NO. 11, which corresponds to the amino acid sequence of the immature PGH isoform NO. 2. This SNP will also be called E210K by reference to the amino acid sequence SEQ ID NO. 11.

The SNP t3436c causes a modification of the PGH protein isoforms encoded by the nucleotide coding sequences SEQ ID NO. 3 and SEQ ID NO. 4. This SNP introduces an arginine (R) instead of a tryptophan (W) (wild-type amino acid) at position 241 of the amino acid sequence SEQ ID NO. 11, which corresponds to the amino acid sequence of the immature PGH isoform NO. 2. This SNP introduces a threonine (T) instead of a methionine (M) (wild-type amino acid) at position 155 of the amino acid sequence SEQ ID NO. 12, which corresponds to the amino acid sequence of the immature PGH isoform NO. 3. This SNP will also be called W241R by reference to amino acid sequence SEQ ID NO. 11, or M155T by reference to amino acid sequence SEQ ID NO. 12. The SNP t3436c does not cause a modification of the PGH protein isoforms encoded by the nucleotide coding sequences SEQ ID NO. 2 and SEQ ID NO. 5. This SNP maintains an aspartic acid (D) at position 156 of the amino acid sequence SEQ ID NO. 10 and at position 141 of the amino acid sequence SEQ ID NO. 13, which correspond to the amino acid sequences of the immature PGH isoform NO. 1 and variant NO. 4, respectively.

The SNP a3655c causes a modification of the PGH protein isoform encoded by the coding nucleotide sequence SEQ ID NO. 4. This SNP introduces a proline (P) instead of a glutamine (Q) (wild-type amino acid) at position 228 of the amino acid sequence SEQ ID NO. 12, which corresponds to the amino acid sequence of the immature PGH isoform NO. 3. This SNP will also be called Q228P by reference to amino acid sequence SEQ ID NO. 12.

The SNP c3693a causes a modification of the PGH protein isoform encoded by the coding nucleotide sequence SEQ ID NO. 4. This SNP introduces a threonine (T) instead of a proline (P) (wild-type amino acid) at position 241 of the amino acid sequence SEQ ID NO. 12, which corresponds to the amino acid sequence of the immature PGH isoform NO. 3. This SNP will also be called P241T by reference to amino acid sequence SEQ ID NO. 12.

The SNP g2435a does not cause a modification of the PGH protein isoforms encoded by the coding nucleotide sequences SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, and SEQ ID NO. 5. This SNP maintains a threonine (T) at position 7 of the amino acid sequences SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12, and SEQ ID NO. 13, which correspond to the amino acid sequences of the immature PGH isoforms NO. 1, NO. 2, NO. 3, and NO. 4, respectively.

The SNP g3267c does not cause a modification of the PGH protein isoform encoded by the coding nucleotide sequence SEQ ID NO. 3. This SNP maintains an alanine (A) at position 184 of the amino acid sequence SEQ ID NO. 11, which corresponds to the amino acid sequence of the immature PGH isoform NO. 2.

Other SNPs in conformity with the invention, namely: g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, a2635c, g3267c, do not involve modification of the protein encoded by the nucleotide sequence of the hGH-V gene at the level of the amino acid sequences SEQ ID NO. 10, SEQ ID NO.11, SEQ ID NO. 12, and SEQ ID NO. 13.

The SNP g2435a is silent and the SNPs g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, and a2635c, are non-coding.

The SNP g3267c may be classified as silent regarding the synthesis of the isoform NO. 2, whereas it may be classified as non-coding regarding the synthesis of the PGH isoforms NO. 1, NO. 3, and NO. 4. SNP t3436c may be classified as silent

regarding the synthesis of the PGH isoforms NO. 1 and NO. 4. SNP g3343a may be classified as non-coding regarding the synthesis of the PGH isoforms NO. 1, NO. 3 and NO. 4. SNPs a3655c and c3693a may be classified as non-coding regarding the synthesis of the PGH isoforms NO. 1, NO. 2, and NO. 4.

The SNP C17stop prematurely interrupts the synthesis of isoforms NO. 2, NO. 3, NO. 4 and NO. 5, leading to a deficiency in mature placental growth hormone.

The SNPs E210K and W241R on the isoform NO. 2 cause modifications of the spatial conformation of the polypeptides in conformity with the invention compared to the polypeptide encoded by the coding nucleotide sequence of the wild-type PGH isoform NO. 2.

These modifications can be predicted by sequence analysis, secondary structure prediction based on the physico-chemical properties of the amino acids, for example, or observed by computational molecular modeling according to methods that are well known to a person skilled in the art making use of, for example, the modeling tools fold recognition (for example, SEQFOLD/MSI), homology (for example, MODELER/MSI), electrostatic fields (DELPHI/MSI), and/or molecular simulation (using force field to determine minimum energy conformations as well as dynamic trajectories of molecular systems using, for example, DISCOVER/MSI).

Sequence analysis and secondary structure predictions show that the mutation E210K on the PGH isoform NO. 2 causes a change in its structure. Indeed, the glutamic acid residue (E) at position 210 of the amino acid sequence of isoform NO. 2 is located in the long coiled region, in particular it is situated between 2 pairs of glycine residues that make this segment very mobile. The SNP E210K may contribute to a structure change since it inverts the residue charge: changing a negatively charged residue (glutamic acid) into a positively charged residue (lysine). The glutamic acid residue at position 210 is conserved in two homologous protein sequences: in the bovine GH variant (Accession number: A29864 in the PIR database) and in the human somatommatropin hormone 1 isoform 2 (Accession number: NP_072166 in GenBank). Thus, the E210K mutated protein possesses a three-dimensional conformation that is different from that of wild-type PGH isoform NO. 2 involving a significant change in its structure and function.

Sequence analysis and secondary structure predictions show that the mutation W241R on the PGH isoform NO. 2 causes a change in its structure. Compared to wild-type PGH isoform NO. 1 and to pituitary GH protein structures, the first 134 amino acids of wild-type PGH isoform NO. 2 share the same three structural alpha-helices, whereas the C-terminal part of the protein is different, containing glycine, proline and serine rich regions. The C-terminal part of the wild-type PGH isoform NO. 2 comprises a long coiled region (70 amino acids) and two short hydrophobic helices, which may be anchored in the cell membrane as suggested by Untergasser et al. (European Journal of Endocrinology (1998) 139:424-427). The residue situated at position 241 on the amino acid sequence of the PGH isoform NO. 2 is located between the two short C-terminal helices of the protein. The facts that tryptophan is an uncommon residue, that the side chain of this residue may interact with other aromatic residues such as F235 or W246, and that the exchange of a tryptophan (W) by an arginine (R) results in a positively charge gain, indicate that the W241R mutation may contribute to a structural change in the mutated PGH isoform NO. 2.

Thus, the W241R mutated PGH isoform 2 possesses a three-dimensional conformation different from the natural wild-type PGH involving a significant change in its structure and function.

Genotyping of the polynucleotides of the invention containing the SNPs of the invention can be carried out in such a way as to determine the allelic frequency of these polynucleotides in a population.

The determination of the functionality of the polypeptides of the invention can equally be carried out by a test of their biological activity.

The invention also has for an object the use of polynucleotides and of polypeptides of the invention for the diagnostic, the prevention and the treatment of certain human disorders and/or diseases.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

By "natural PGH" or "natural wild-type PGH" is understood the mature placental growth hormone (PGH) encoded by the nucleotide sequence of the reference wild-type hGH-V gene.

By "polynucleotide" is understood a polyribonucleotide or a polydeoxyribonucleotide that can be a modified or a non-modified DNA or an RNA.

The term polynucleotide includes, for example, a single strand or double strand DNA, a DNA composed of a mixture of one or several single strand region(s) and of one or several double strand region(s), a single strand or double strand RNA and an RNA composed of a mixture of one or several single strand region(s) and of one or several double strand region(s). The term polynucleotide can also include an RNA and/or a DNA including one or several triple strand regions. By polynucleotide is equally understood the DNAs and RNAs containing one or several bases modified in such a fashion as to have a skeleton modified for reasons of stability or for other reasons. By modified base is understood, for example, the unusual bases such as inosine.

By "polypeptide" is understood a peptide, an oligopeptide, an oligomer or a protein comprising at least two amino acids joined to each other by a normal or modified peptide bond, such as in the cases of the isosteric peptides, for example.

A polypeptide can be composed of amino acids other than the 20 amino acids defined by the genetic code. A polypeptide can equally be composed of amino acids modified by natural processes, such as post translational maturation processes or by chemical processes, which are well known to a person skilled in the art. Such modifications are fully detailed in the literature. These modifications can appear anywhere in the polypeptide: in the peptide skeleton, in the amino acid chain or even at the carboxy- or amino-terminal ends.

A polypeptide can be branched following an ubiquitination or be cyclic with or without branching. This type of modification can be the result of natural or synthetic post-translational processes that are well known to a person skilled in the art.

For example, by polypeptide modifications is understood acetylation, acylation, ADP-ribosylation, amidation, covalent fixation of flavine, covalent fixation of heme, covalent fixation of a nucleotide or of a nucleotide derivative, covalent fixation of a lipid or of a lipidic derivative, the covalent fixation of a phosphatidylinositol, covalent or non-covalent cross-linking, cyclization, disulfide bridge formation, demethylation, cysteine formation, pyroglutamate formation, formylation, gamma-carboxylation, glycosylation, PEGylation, GPI anchor formation, hydroxylation, iodization, methylation, myristoylation, oxidation, proteolytic processes, phosphorylation, prenylation, racemization, seneloylation, sulfatation, amino acid addition such as arginylation or ubiquitination. Such modifications are fully detailed in the literature: PROTEINS-STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, New York, 1993, POST-TRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983, Seifter et al. "Analysis for protein modifications and nonprotein cofactors", Meth. Enzymol. (1990) 182: 626-646 et Rattan et al. "Protein Synthesis: Post-translational Modifications and Aging", Ann NY Acad Sci (1992) 663: 48-62.

By "isolated polynucleotide" or "isolated polypeptide" is understood a polynucleotide or a polypeptide such as previously defined which is isolated from the human body or otherwise produced by a technical process.

By "identity" is understood the measurement of nucleotide or polypeptide sequence identity. Identity is a term well known to a person skilled in the art and well described in the literature. See COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., Ed., Oxford University Press, New York, 1998; BIOCOMPUTING INFORMATICS AND GENOME PROJECT, Smith, D.W., Ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M. and Griffin H.G., Ed, Humana Press, New Jersey, 1994; et SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987.

The methods commonly employed to determine the identity and the similarity between two sequences are equally well described in the literature. See GUIDE TO HUGE COMPUTER, Martin J. Bishop, Ed, Academic Press, San Diego, 1994, et Carillo H. and Lipton D., Siam J Applied Math (1988) 48: 1073.

A polynucleotide having, for example, an identity of at least 95 % with the nucleotide sequence SEQ ID NO. 1 is a polynucleotide which contains at most 5 points of mutation over 100 nucleotides, compared to said sequence.

These points of mutation can be one (or several) substitution(s), addition(s) and/or deletion(s) of one (or several) nucleotide(s).

In the same way, a polypeptide having, for example, an identity of at least 95 % with the amino acid sequence SEQ ID NO. 7 is a polypeptide that contains at most 5 points of mutation over 100 amino acids, compared to said sequence.

These points of mutation can be one (or several) substitution(s), addition(s) and/or deletion(s) of one (or several) amino acid(s).

The polynucleotides and the polypeptides according to the invention which are not totally identical with respectively the nucleotide sequence SEQ ID NO. 1 or the amino acid sequence SEQ ID NO. 7, it being understood that these sequences contain at least one of the SNPs of the invention, are considered as variants of these sequences.

A variant, according to the invention, can be obtained, for example, by site-directed mutagenesis or by direct synthesis.

By "SNP" (Single Nucleotide Polymorphism) is understood any natural variation of a base in a nucleotide sequence. A SNP, on a nucleotide sequence, can be coding, silent or non-coding.

A coding SNP is a polymorphism included in the coding sequence of a nucleotide sequence that involves a modification of an amino acid in the sequence of amino acids encoded by this nucleotide sequence. In this case, the term SNP applies equally, by extension, to a mutation in an amino acid sequence.

A silent SNP is a polymorphism included in the coding sequence of a nucleotide sequence that does not involve a modification of an amino acid in the amino acid sequence encoded by this nucleotide sequence.

A non-coding SNP is a polymorphism included in the non-coding sequence of a nucleotide sequence. This polymorphism can notably be found in an intron, a splicing zone, a transcription promoter or a site enhancer sequence.

Polynucleotide

The present invention has for its first object an isolated polynucleotide comprising:

a) A nucleotide sequence having at least 95 % identity, preferably at least 97 % identity, more preferably at least 99 % identity with the nucleotide sequence SEQ ID NO. 1, provided that such nucleotide sequence comprises at least one SNP selected from the group consisting of g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c, and c3693a; or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

The present invention has for an object an isolated polynucleotide comprising:

a) A nucleotide sequence having at least 95 % identity, preferably at least 97 % identity, more preferably at least 99 % identity with the nucleotide sequence SEQ ID NO. 2, provided that said sequence contains at least one coding SNP selected from the group consisting of c51a, and t454g; or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

The present invention also has for an object an isolated polynucleotide comprising a nucleotide sequence having at least 95 % identity, preferably at least 97 % identity, more preferably at least 99 % identity with:

a) The nucleotide sequence SEQ ID NO. 3, provided that said sequence contains at least one coding SNP selected from the group consisting of c51a, t454g, g628a, and t721c; or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

The present invention also has for an object an isolated polynucleotide comprising a nucleotide sequence having at least 95 % identity, preferably at least 97 % identity, more preferably at least 99 % identity with:

a) The nucleotide sequence SEQ ID NO. 4, provided that said sequence contains at least one coding SNP selected from the group consisting of c51a, t464c, a683c, and c721a; or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

The present invention also has for an object an isolated polynucleotide comprising a nucleotide sequence having at least 95 % identity, preferably at least 97 %

identity, more preferably at least 99 % identity with:

a) The nucleotide sequence SEQ ID NO. 5, provided that said sequence contains at least one coding SNP selected from the group consisting of c51a, and t409g; or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

The present invention relates equally to an isolated polynucleotide comprising:

a) All or part of the nucleotide sequence SEQ ID NO. 1, provided that such nucleotide sequence, or part of sequence, comprises at least one SNP selected from the group consisting of g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c, and c3693a; or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

Preferably, according to the invention the polynucleotide previously defined comprises a single SNP selected from the group consisting of: c2465a, t3169g, g3343a, t3436c, a3655c, and c3693a.

More preferably, the polynucleotide previously defined comprises the coding SNP c2465a.

The present invention also relates to an isolated polynucleotide comprising:

a) All or part of the nucleotide sequence SEQ ID NO. 2, provided that said sequence, or part of sequence, contains at least one coding SNP selected from the group consisting of c51a, and t454g; or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

The present invention also relates to an isolated polynucleotide comprising:

a) All or part of the nucleotide sequence SEQ ID NO. 3, provided that said sequence, or part of sequence, contains at least one coding SNP selected from the group consisting of c51a, t454g, g628a, and t721c; or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

The present invention also relates to an isolated polynucleotide comprising:

a) All or part of the nucleotide sequence SEQ ID NO. 4, provided that said sequence, or part of sequence, contains at least one coding SNP selected from the group consisting of c51a, t464c, a683c, and c721a; or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

The present invention also relates to an isolated polynucleotide comprising:

a) All or part of the nucleotide sequence SEQ ID NO. 5, provided that said sequence, or part of sequence, contains at least one coding SNP selected from the group consisting of c51a, and t409g; or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

Said isolated polynucleotides are composed of at least 10 nucleotides.

Preferably, the polynucleotides as defined above are composed of 10 to 40 nucleotides.

Preferably, the polynucleotide of the invention consists of:

a) All or part of the nucleotide sequence SEQ ID NO. 1, provided that said sequence, or part of sequence, contains at least one SNP selected from the group consisting of g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c, and c3693a; or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

Preferably, the polynucleotide of the invention consists of:

a) All or part of the nucleotide coding sequence SEQ ID NO. 2, provided that said sequence, or part of sequence, contains at least one coding SNP selected from the group consisting of c51a, and t454g; or

b) All or part of the nucleotide coding sequence SEQ ID NO. 3, provided that said sequence, or part of sequence, contains at least one coding SNP selected from the group consisting of c51a, t454g, g628a, and t721c; or

c) All or part of the nucleotide coding sequence SEQ ID NO. 4, provided that said sequence, or part of sequence, contains at least one coding SNP selected from the group consisting of c51a, t464c, a683c, and c721a; or

d) All or part of the nucleotide coding sequence SEQ ID NO. 5, provided that said sequence, or part of sequence, contains at least one coding SNP selected from the group consisting of c51a, and t409g; or

e) A nucleotide sequence complementary to a nucleotide sequence under a) to d).

said isolated polynucleotide being composed of at least 10 nucleotides.

Preferably, the isolated polynucleotide as defined above is composed of 10 to 40 nucleotides.

A polynucleotide such as previously defined can equally include at least one of the following silent SNPs:

a) g10a, t468c in nucleotide sequence SEQ ID NO. 2; or

b) g10a, g552c in nucleotide sequence SEQ ID NO. 3; or

c) g10a in nucleotide sequence SEQ ID NO. 4; or

d) g10a, t423c in nucleotide sequence SEQ ID NO. 5.

The present invention equally has for its object an isolated polynucleotide comprising or consisting of:

a) All or part of a nucleotide sequence SEQ ID NO. 2, it being understood that this sequence, or part of sequence, comprises at least one of the following silent SNPs: g10a, t468c, or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

The present invention equally has for its object an isolated polynucleotide comprising or consisting of:

a) All or part of a nucleotide sequence SEQ ID NO. 3, it being understood that this sequence, or part of sequence, comprises at least one of the following silent SNPs: g10a, g552c, or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

The present invention equally has for its object an isolated polynucleotide comprising or consisting of:

a) All or part of a nucleotide sequence SEQ ID NO. 4, it being understood that this sequence, or part of sequence, comprises the following silent SNPs: g10a, or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

The present invention equally has for its object an isolated polynucleotide comprising or consisting of:

a) All or part of a nucleotide sequence SEQ ID NO. 5, it being understood that this sequence, or part of sequence, comprises at least one of the following silent SNPs: g10a, t423c, or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

The isolated polynucleotides as defined above are composed of at least 10 nucleotides, preferably from 10 to 40 nucleotides.

The present invention also concerns an isolated polynucleotide consisting of:

a) The nucleotide sequence SEQ ID NO. 6, or

b) A nucleotide sequence comprising the nucleotide sequence under a) and at most 100 additional nucleotides, and preferably at most 50 additional nucleotides, at the 5' end, or at the 3' end, or at each of the 5' and 3' ends of said nucleotide sequence under a) ; or

c) A nucleotide sequence complementary to one of the nucleotide sequences under a) or b).

The present invention also has for its object an isolated polynucleotide encoding for a polypeptide consisting of the amino acid sequence SEQ ID NO. 7.

The present invention also has for its object an isolated polynucleotide coding for a polypeptide comprising all or part of the amino acid sequence SEQ ID NO. 7.

More preferably, the present invention has for an object a chimeric polypeptide comprising the amino acid sequence SEQ ID NO. 7.

The present invention also has for its object an isolated polynucleotide coding for a polypeptide comprising all or part of:

a) the amino acid sequence SEQ ID NO. 10, or

b) the amino acid sequence comprising the amino acids included between positions 27 and 217 of the sequence of amino acids SEQ ID NO. 10,

it being understood that said polypeptide comprises the coding SNP: W152G.

It is understood, in the sense of the present invention, that the numbering corresponding to the positioning of the coding SNP W152G is relative to the numbering of the amino acid sequence SEQ ID NO. 10.

The present invention also has for its object an isolated polynucleotide coding for a polypeptide comprising all or part of:

a) the amino acid sequence SEQ ID NO. 11, or

b) the amino acid sequence comprising the amino acids included between positions 27 and 256 of the sequence of amino acids SEQ ID NO. 11,

it being understood that said polypeptide comprises at least one of the following coding SNPs: W152G, E210K, W241R.

It is understood, in the sense of the present invention, that the numbering

corresponding to the positioning of the coding SNPs W152G, E210K, and W241R, is relative to the numbering of the amino acid sequence SEQ ID NO. 11.

The present invention also has for its object an isolated polynucleotide coding for a polypeptide comprising all or part of:

a) the amino acid sequence SEQ ID NO. 12, or

b) the amino acid sequence comprising the amino acids included between positions 27 and 245 of the sequence of amino acids SEQ ID NO. 12,

it being understood that said polypeptide comprises at least one of the following coding SNPs: I151 splice, M155T, Q228P, P241T.

It is understood, in the sense of the present invention, that the numbering corresponding to the positioning of the coding SNPs I151 splice, M155T, Q228P, and P241T, is relative to the numbering of the amino acid sequence SEQ ID NO. 12.

The present invention also has for its object an isolated polynucleotide coding for a polypeptide comprising all or part of:

a) the amino acid sequence SEQ ID NO. 13, or

b) the amino acid sequence comprising the amino acids included between positions 27 and 202 of the sequence of amino acids SEQ ID NO. 13,

it being understood that said polypeptide comprises the coding SNP W137G.

It is understood, in the sense of the present invention, that the numbering corresponding to the positioning of the coding SNP W137G, is relative to the numbering of the amino acid sequence SEQ ID NO. 13.

According to a preferred object of the invention, the previously defined polypeptides comprise a single coding SNP such as defined above.

Preferably a polynucleotide according to the invention is composed of a DNA or RNA molecule.

A polynucleotide according to the invention can be obtained by standard DNA or RNA synthetic methods.

A polynucleotide according to the invention can equally be obtained by site-directed mutagenesis starting from all or part of the nucleotide sequence of the hGH-V gene by modifying the wild-type nucleotide by the mutated nucleotide at the position of the SNP. For example, a polynucleotide according to the invention comprising the SNP

c2465a can be obtained by site-directed mutagenesis starting from all or part of the nucleotide sequence of the hGH-V gene by modifying the wild-type cytosine (c) nucleotide by the mutated adenine (a) nucleotide at position 2465 in SEQ ID NO. 1 (or position 51 in SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4 and/or SEQ ID NO. 5).

The processes of site-directed mutagenesis that can be implemented in this way are well known to a person skilled in the art. The publication of TA Kunkel in 1985 in "Proc. Natl. Acad. Sci. USA" 82:488 can notably be mentioned.

An isolated polynucleotide can equally include, for example, nucleotide sequences coding for pre-, pro- or pre-pro-protein amino acid sequences or marker amino acid sequences, such as hexa-histidine peptide.

A polynucleotide of the invention can equally be associated with nucleotide sequences coding for other proteins or protein fragments in order to obtain fusion proteins or other purification products.

A polynucleotide according to the invention can equally include nucleotide sequences such as the 5' and/or 3' non-coding sequences, such as, for example, transcribed or non-transcribed sequences, translated or non-translated sequences, splicing signal sequences, polyadenylated sequences, ribosome binding sequences or even sequences which stabilize mRNA.

A nucleotide sequence complementary to the nucleotide or polynucleotide sequence is defined as one that can be hybridized with this nucleotide sequence, under stringent conditions.

By "stringent hybridization conditions" is generally but not necessarily understood the chemical conditions that permit a hybridization when the nucleotide sequences have an identity of at least 95 %, preferably greater than or equal to 97 %, still more preferably greater than or equal to 99 % and most preferably equal to 100 %.

The stringent conditions can be obtained according to methods well known to a person skilled in the art and, for example, by an incubation of the polynucleotides, at 42° C, in a solution comprising 50 % formamide, 5xSSC (150 mM of NaCl, 15 mM of trisodium citrate), 50 mM of sodium phosphate (pH = 7.6), 5x Denhardt Solution, 10 % dextran sulfate and 20 µg denatured salmon sperm DNA, followed by washing the filters at 0.1x SSC, at 65° C.

Within the scope of the invention, when the stringent hybridization conditions permit hybridization of the nucleotide sequences having an identity equal to 100 %, the nucleotide sequence is considered to be strictly complementary to the nucleotide sequence such as described under a).

It is understood within the meaning of the present invention that the nucleotide sequence complementary to a nucleotide sequence comprises at least one anti-sense SNP according to the invention. Thus, for example, if the nucleotide sequence comprises the SNP c2465a, its complementary nucleotide sequence comprises the nucleotide thymine (t) at the equivalent of position 2465.

Identification, hybridization and/or amplification of a polynucleotide comprising the SNPs of the invention

The present invention also has for its object the use of all or part of:

a) a polynucleotide having 90 to 100% identity (preferably at least 97 % identity, more preferably 99 % identity and particularly 100% identity) with nucleotide sequence SEQ ID NO. 1; or

b) a polynucleotide according to the invention comprising at least one SNP,

in order to identify, hybridize, and/or amplify all or part of a polynucleotide having 90 to 100% (preferably at least 97 % identity, more preferably 99 % identity and particularly 100% identity) identity with nucleotide sequence SEQ ID NO. 1 or if necessary its coding sequence,

it being understood that each one of these sequences comprises at least one of the following SNPs: g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c, c3693a.

The present invention concerns a method for identifying or amplifying all or part of a polynucleotide having 90 to 100% identity with nucleotide sequence SEQ ID NO. 1, comprising hybridizing under appropriate hybridization conditions said polynucleotide with a polynucleotide according to the invention.

Genotyping and determination of the frequency of a SNP

The present invention equally has for its object the use of all or part of:

a) a polynucleotide having 90 to 100 % identity (preferably at least 97 % identity, more preferably 99 % identity and particularly 100% identity) with nucleotide sequence SEQ ID NO. 1; or

b) a polynucleotide according to the invention comprising at least one SNP,

for the genotyping of all or part of a polynucleotide having 90 to 100% identity (preferably at least 97 % identity, more preferably 99 % identity and particularly 100% identity) with nucleotide sequence SEQ ID NO. 1 or if necessary its coding sequence,

it being understood that each one of these sequences comprises at least one of the following SNPs: g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c, c3693a.

Preferably, the present invention has for its object the use of all or part of a polynucleotide of the invention for the genotyping of a nucleotide sequence which has 90 to 100 % identity (preferably at least 97 % identity, more preferably 99 % identity and particularly 100% identity) with the nucleotide sequence of a growth hormone gene and which comprises the SNP c2465a (or c51a).

Said growth hormone gene can be placental growth hormone gene (hGH-V) and/or the adult growth hormone gene (hGH-N).

The present invention concerns a method for genotyping all or part of a polynucleotide having 90 to 100% identity with nucleotide sequence SEQ ID NO. 1, comprising the steps of amplifying a region of interest in the genomic DNA of a subject or a population of subjects, and determining the allele of at least one position in the nucleotide sequence SEQ ID NO. 1 chosen from the group consisting of: 2139, 2197, 2204, 2205, 2206, 2267, 2435, 2465, 2635, 3169, 3267, 3343, 3436, 3655 and 3693.

Within the meaning of the invention, genotyping is defined as a process for the determination of the genotype of an individual or of a population of individuals. Genotype consists of the alleles present at one or more specific loci.

By "population of individuals" is understood a group of determined individuals selected in random or non-random fashion. These individuals can be humans, animals, microorganisms or plants.

Usually, the group of individuals comprises at least 10 persons, preferably from 100 to 300 persons.

The individuals can be selected according to their ethnicity or according to their phenotype, notably those who are affected by the following disorders and/or diseases involving the human growth and development, such as fetal growth and development, perinatal carbohydrate metabolism, phallic growth, craniofacial developments, hypochondroplasia, Laron type of dwarfism ; disorders related to IGF-1 secretion, such as cognitive functions reduction, mental retardation, sensorineural deafness, insulin resistance, type II diabete, hematological disorders ; tumors and cancers such as breast cancer and prostate cancer ; disorders or diseases linked to the immune system such as allergies, auto-immune diseases, graft rejection, and certain infectious diseases ; metabolic disorders or diseases related to lipid, nitrogen and/or carbohydrate metabolism such as obesity, arteriosclerosis, body mass maintenance ; disorders or diseases linked to angiogenesis, retinopathy, cardiovascular diseases.

Multiple technologies exist which can be implemented in order to genotype SNPs (see notably Kwok Pharmacogenomics, 2000, vol 1, pp 95-100. "High–throughput genotyping assay approaches"). These technologies are based on one of the four following principles: allele specific oligonucleotide hybridization, oligonucleotide elongation by dideoxynucleotides optionally in the presence of deoxynucleotides, ligation of allele specific oligonucleotides or cleavage of allele specific oligonucleotides. Each one of these technologies can be coupled to a detection system such as measurement of direct or polarized fluorescence, or mass spectrometry.

Genotyping can notably be carried out by minisequencing with hot ddNTPs (2 different ddNTPs labeled by different fluorophores) and cold ddNTPs (2 non labeled ddNTPs), in connection with a polarized fluorescence scanner. The minisequencing protocol with reading of polarized fluorescence (Technology FP-TDI or Fluorescence Polarization Template-direct Dye-Terminator Incorporation) is well known to a person skilled in the art.

It can be carried out on a product obtained after amplification by polymerase chain reaction (PCR) of the DNA of each individual. This PCR product is selected to cover the polynucleotide genic region containing the studied SNP.

The sense and antisense primers, respectively, for the PCR amplification, in the case of a SNP of the invention, can easily be selected by a person skilled in the art

according to the position of the SNP according to the invention.

For example, the sense and antisense nucleotide sequences for the PCR amplification of a fragment comprising SNP c2465a can be, respectively, SEQ ID NO. 8 and SEQ ID NO. 9. These nucleotide sequences permit amplification of a fragment having a length of 351nucleotides in the nucleotide sequence SEQ ID NO. 1.

More specifically, other examples of sense and antisense nucleotide sequences for the PCR amplification of a fragment comprising the SNPs of the invention may be the following:

SNPs	Sense and antisense primers	Length of the fragment (nucleo-tides)
g2139c, g2197t, g2204a, g2205t, c2206t, g2267a	Sense: SEQ ID NO. 14: CAGGTGAGGAGAAGCAGCGA Antisense: SEQ ID NO. 15: CTGCCTCCCTCCAGGGAC	312
g2435a, c2465a, a2635c	Sense: SEQ ID NO. 16: GTCCCTGGAGGGAGGCAGA Antisense: SEQ ID NO. 17: CTGCCTGCATTTTCACTTCAGAA	400
t3169g, g3267c, g3343a, t3436c	Sense: SEQ ID NO. 18: AACGTCTATCGCCACCTG Antisense: SEQ ID NO. 19: GTCAAACTTGCTGTAGGACTGA	369
t3436c, a3655c	Sense: SEQ ID NO. 20: CCAGGCCTTTCTCTACAACCTGG Antisense: SEQ ID NO. 21: CTGGAGTAGCACCTTCCACGA	374
c3693a	Sense: SEQ ID NO. 22: GGCAGATCTTCAATCAGT Antisense: SEQ ID NO. 23: TTATACAAGGACACCTAGTCAA	304

After elimination of the PCR primers that have not been used and the dNTPs that have not been incorporated, the minisequencing is carried out. The minisequencing may consist of lengthening an oligonucleotide primer, placed just upstream of the site of the SNP, by using a polymerase enzyme and fluorolabeled dideoxynucleotides, for example. The product resulting from this lengthening process may be directly analyzed by a reading of polarized fluorescence. After the last stage in the PCR thermocycler, the plate may be placed on a polarized fluorescence scanner for a reading of the labeled bases by using fluorophore specific excitation and emission filters. The intensity values of the labeled bases are reported on a graph and used to determine the genotype.

A statistical analysis of the frequency of each allele (allelic frequency) encoded by the gene comprising the SNP in the population of individuals is then achieved, which permits determination of the importance of their impact and their distribution in the

different sub-groups and notably, if necessary, the diverse ethnic groups that constitute this population of individuals.

The genotyping data are analyzed in order to estimate the distribution frequency of the different alleles observed in the studied populations. The calculations of the allelic frequencies can be carried out with the help of software such as SAS-suite® (SAS) or SPLUS® (MathSoft). The comparison of the allelic distributions of a SNP of the invention across different ethnic groups of the population of individuals can be carried out by means of the software ARLEQUIN® and SAS-suite®.

SNPs of the invention as genetic markers.

Whereas SNPs modifying functional sequences of genes (e.g. promoter, splicing sites, coding region) are likely to be directly related to disease susceptibility or resistance, all SNPs (functional or not) may provide valuable markers for the identification of one or several genes involved in these disease states and, consequently, may be indirectly related to these disease states (See Cargill et al. (1999). Nature Genetics 22:231-238; Riley et al. (2000). Pharmacogenomics 1:39-47; Roberts L. (2000). Science 287: 1898-1899).

Thus, the present invention also concerns a databank comprising at least one of the following SNPs: g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c, c3693a, in a polynucleotide of the hGH-V gene.

It is well understood that said SNPs are numbered in accordance with the nucleotide sequence SEQ ID NO. 1.

This databank may be analyzed for determining statistically relevant associations between:

(i) at least one of the following SNPs: g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c, c3693a, in a polynucleotide of the hGH-V gene, and

(ii) a disease or a resistance to a disease.

More preferably, the present invention concerns a method for determining statistically relevant associations between at least one SNP selected from the group

consisting of g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c, and c3693a, in a polynucleotide of the hGH-V gene, and a disease or resistance to disease comprising:

a) Genotyping a group of individuals;

b) Determining the distribution of said disease or resistance to disease within said group of individuals;

c) Comparing the genotype data with the distribution of said disease or resistance to disease; and

d) Analyzing said comparison for statistically relevant associations.

A SNP of the invention such as defined above may be directly or indirectly associated to a disease or a resistance to a disease.

Preferably, these diseases may be those which are defined as mentioned above.

Expression vector and host cell

The present invention also has for its object a recombinant vector comprising at least one polynucleotide according to the invention.

Numerous expression systems can be used, like, for example, chromosomes, episomes, derived viruses. More particularly, the recombinant vectors used can be derived from bacterial plasmids, transposons, yeast episome, insertion elements, yeast chromosome elements, viruses such as baculovirus, papilloma viruses such as V40, vaccinia viruses adenoviruses, fox pox viruses, pseudorabies viruses, retroviruses.

These recombinant vectors can equally be cosmid or phagemid derivatives. The nucleotide sequence can be inserted in the recombinant expression vector by methods well known to a person skilled in the art such as, for example, those that are described in MOLECULAR CLONING, A LABORATORY MANUAL (supra) Sambrook et al.

The recombinant vector can include nucleotide sequences that control the regulation of the polynucleotide expression as well as nucleotide sequences permitting the expression and the transcription of a polynucleotide of the invention and the translation of a polypeptide of the invention, these sequences being selected according to the host cells that are used.

Thus, for example, an appropriate secretion signal can be integrated in the recombinant vector so that the polypeptide, encoded by the polynucleotide of the invention, will be directed towards the opening of the endoplasmic reticulum, towards the periplasmic space, on the membrane or towards the extracellular environment.

The present invention also has for its object a host cell comprising a recombinant vector according to the invention.

The introduction of the recombinant vector in a host cell can be carried out according to methods that are well known to a person skilled in the art such as those described in BASIC METHODS IN MOLECULAR BIOLOGY, Davis et al., 1986 et MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989, such as transfection by calcium phosphate, transfection by DEAE dextran, transfection, microinjection, transfection by cationic lipids, electroporation, transduction or infection.

The host cell can be, for example, bacterial cells such as cells of streptococci, staphylococci, *E. coli* or *Bacillus subtilis*, cells of fungi such as yeast cells and cells of *Aspergillus, Streptomyces*, insect cells such as cells of *Drosophila* S2 and of *Spodoptera* Sf9, animal cells, such as CHO, COS, HeLa, C127, BHK, HEK 293 cells and human cells of the subject to treat or even plant cells.

The host cells can be used, for example, to express a polypeptide of the invention or as an active product in pharmaceutical compositions, as will be seen hereinafter.

Polypeptide

The present invention also has for its object an isolated chimeric polypeptide comprising an amino acid sequence having at least 95 % identity, preferably at least 97 % identity, more preferably at least 99 % identity, with the amino acid sequence SEQ ID NO. 7.

The chimeric polypeptide of the invention can equally comprise the amino acid sequence SEQ ID NO. 7.

By "chimeric polypeptide" is understood a polypeptide resulting from the fusion of at least two polypeptides which are not naturally found linked together in an organism.

A chimeric polypeptide can be obtained by chemical cross-linking, which can be accomplished by means of bifunctional linker molecules which react with amino groups of the polypeptides or cysteine residues (Fanger M.W. et al. (1992). Crit. Rev. Immunol. 12: 101-124).

Methods based on genetic engineering may constitute another mean to produce a chimeric polypeptide resulting from the ligation of two nucleotide sequences. For example, the 5' end of the coding sequence of a protein may be linked with the 3' end of the coding sequence of another protein by genetic engineering methods, while the reading frame is retained, and the construct is expressed recombinantly in prokaryotes or eukaryotes. Chimeric polypeptide can be made according to methods well known to a person skilled in the art such as those described in: Ed. Ausubel, Brent et al.; in Current protocols in molecular biology (1997); Chapter 16: protein expression; Higuchi et al.; Nuc. Acids Res.; 16 : 7351-7367 (1988), Clackson et al.; Nuc. Acids Res.; 17 : 10163-10170 (1989), Blaschke et al. (2000). Protein engineering by expressed protein ligation. Methods Enzymol. 328:478-96; Buck K.J. and Amara S.G. (1998). In vivo generation of chimeras. Methods Enzymol. 296:466-75.; Volkov A.A. and Arnold F.H. (2000). Methods for in vitro DNA recombination and random chimeragenesis. Methods Enzymol. 328:447-56 and Fishbein et al. (1994). A chimeric VIP-PACAP analogue but not VIP pseudopeptides function as VIP receptor antagonists. Peptides. 15:95-100.

The present invention concerns also a polypeptide consisting of an amino acid sequence having at least 95 % identity, preferably at least 97 % identity, more preferably at least 99 % identity, with the amino acid sequence SEQ ID NO. 7.

Preferably, the polypeptide of the invention consists of the amino acid sequence SEQ ID NO. 7.

The present invention concerns also a polypeptide comprising an amino acid sequence having at least 95 % identity, preferably at least 97 % identity, more preferably at least 99 % identity, with all or part of:

a) the amino acid sequence SEQ ID NO. 10, or

b) the amino acid sequence comprising amino acids 27 through 217 of the amino acid sequence SEQ ID NO. 10,

it being understood that said polypeptide contains the coding SNP W152G.

The polypeptide of the invention can equally comprise all or part of:

a) the amino acid sequence SEQ ID NO. 10, or

b) the amino acid sequence comprising amino acids 27 through 217 of the amino acid sequence SEQ ID NO. 10,

it being understood that said polypeptide contains the coding SNP W152G.

The present invention can more particularly consist of all or part of:

a) the amino acid sequence SEQ ID NO. 10, or

b) the amino acid sequence comprising amino acids 27 through 217 of the amino acid sequence SEQ ID NO. 10,

it being understood that said polypeptide contains the coding SNP W152G.

The present invention concerns also a polypeptide comprising an amino acid sequence having at least 95 % identity, preferably at least 97 % identity, more preferably at least 99 % identity, with all or part of:

a) the amino acid sequence SEQ ID NO. 11, or

b) the amino acid sequence comprising amino acids 27 through 256 of the amino acid sequence SEQ ID NO. 11,

it being understood that said polypeptide contains at least one of the following coding SNPs: W152G, E210K, W241R.

The polypeptide of the invention can equally comprise all or part of:

a) the amino acid sequence SEQ ID NO. 11, or

b) the amino acid sequence comprising amino acids 27 through 256 of the amino acid sequence SEQ ID NO. 11,

it being understood that said polypeptide contains at least one of the following coding SNPs: W152G, E210K, W241R.

The present invention can more particularly consist of all or part of:

a) the amino acid sequence SEQ ID NO. 11, or

b) the amino acid sequence comprising amino acids 27 through 256 of the amino acid sequence SEQ ID NO. 11,

it being understood that said polypeptide contains at least one of the following coding SNPs: W152G, E210K, W241R.

Preferably, a polypeptide according to the invention contains a single coding SNP selected from the group consisting of: W152G, E210K, and W241R.

The present invention concerns also a polypeptide comprising an amino acid sequence having at least 95 % identity, preferably at least 97 % identity, more preferably at least 99 % identity, with all or part of:

a) the amino acid sequence SEQ ID NO. 12, or

b) the amino acid sequence comprising the amino acids 27 through 245 of the amino acid sequence SEQ ID NO. 12,

it being understood that said polypeptide contains at least one of the following coding SNPs: I151splice, M155T, Q228P, P241T.

The polypeptide of the invention can equally comprise all or part of:

a) the amino acid sequence SEQ ID NO. 12, or

b) the amino acid sequence comprising the amino acids 27 through 245 of the amino acid sequence SEQ ID NO. 12,

it being understood that said polypeptide contains at least one of the following coding SNPs: I151splice, M155T, Q228P, P241T.

The present invention can more particularly consist of all or part of:

a) the amino acid sequence SEQ ID NO. 12, or

b) the amino acid sequence comprising the amino acids 27 through 245 of the amino acid sequence SEQ ID NO. 12,

it being understood that said polypeptide contains at least one of the following coding SNPs: I151splice, M155T, Q228P, and P241T.

Preferably, a polypeptide according to the invention contains a single coding SNP selected from the group consisting of: I151splice, M155T, Q228P, and P241T.

The present invention concerns also a polypeptide comprising an amino acid sequence having at least 95 % identity, preferably at least 97 % identity, more preferably at least 99 % identity, with all or part of:

a) the amino acid sequence SEQ ID NO. 13, or

b) the amino acid sequence comprising the amino acids 27 through 217 of the amino acid sequence SEQ ID NO. 13,

it being understood that said polypeptide contains the coding SNP W137G.

The polypeptide of the invention can equally comprise all or part of:

a) the amino acid sequence SEQ ID NO. 13, or

b) the amino acid sequence comprising the amino acids 27 through 217 of the amino acid sequence SEQ ID NO. 13,

it being understood that said polypeptide contains the coding SNP W137G.

The present invention can more particularly consist of all or part of:

a) the amino acid sequence SEQ ID NO. 13, or

b) the amino acid sequence comprising amino acids 27 through 202 of the amino acid sequence SEQ ID NO. 13,

it being understood that said polypeptide contains the coding SNP W137G.

The present invention has for an object the polypeptide encoded by an isolated polynucleotide comprising all or part of the nucleotide sequence SEQ ID NO. 1 provided that such nucleotide sequence, or part of sequence, comprises at least one SNP selected from the group consisting of g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c and c3693a.

Preferably, the polypeptide of the invention is encoded by all or part of the nucleotide sequence SEQ ID NO. 1, provided that said sequence or part thereof comprises the SNP t3169g.

The invention also concerns an isolated polypeptide which is encoded by a nucleotide sequence selected in the group consisting of:

a) a nucleotide sequence SEQ ID NO. 2; or

b) a part of nucleotide sequence SEQ ID NO. 2; or

c) a nucleotide sequence SEQ ID NO. 3; or

d) a part of nucleotide sequence SEQ ID NO. 3,

provided that the sequences under a), b), c) or d) comprise the SNP t454g.

The invention equally concerns an isolated polypeptide which is encoded by all or part of the nucleotide sequence SEQ ID NO. 5, provided that said sequence or said part thereof comprises the SNP t409g.

The present invention equally has for its object a process for the preparation of the above-described polypeptide, in which a previously defined host cell is cultivated in a culture medium and said polypeptide is isolated from the culture medium.

The polypeptide can be purified starting from the host cells, according to methods well known to a person skilled in the art such as precipitation with the help of chaotropic agents such as salts, in particular ammonium sulfate, ethanol acetone or trichloroacetic acid, acid extraction; ion exchange chromatography; phosphocellulose chromatography; hydrophobic interaction chromatography; affinity chromatography; hydroxyapatite chromatography or exclusion chromatographies.

By "culture medium" is understood the medium in which the polypeptide of the invention is isolated or purified. This medium can be composed of the extracellular medium and/or the cellular lysate. Techniques well known to a person skilled in the art equally permit the latter to give back an active conformation to the polypeptide, if the conformation of said polypeptide was altered during the isolation or the purification.

Antibodies

The present invention also has for its object a process for obtaining an immunospecific antibody.

By "antibody" is understood the monoclonal, polyclonal, chimeric, simple chain, humanized antibodies as well as the Fab fragments, including Fab or immunoglobulin expression library products.

An immunospecific antibody can be obtained by immunization of an animal with a polypeptide according to the invention and the recovery of the immunospecific antibody.

The invention also relates to an immunospecific antibody for a polypeptide according to the invention, such as defined previously.

A polypeptide according to the invention, one of its fragments, an analog, one of its variants or a cell expressing this polypeptide can also be used to produce immunospecific antibodies.

The term "immunospecific" means that the antibody possesses a better affinity for the polypeptide of the invention than for other polypeptides known in the prior art.

The immunospecific antibodies can be obtained by administration of a polypeptide of the invention, of one of its fragments, of an analog or of an epitopic fragment or of a cell expressing this polynucleotide in a mammal, preferably non human, according to methods well known to a person skilled in the art.

For the preparation of monoclonal antibodies, typical methods for antibody production can be used, starting from cell lines, such as the hybridoma technique (Kohler et al., Nature (1975) 256: 495-497), the trioma technique, the human B cell hybridoma technique (Kozbor et al., Immunology Today (1983) 4: 72) and the EBV hybridoma technique (Cole et al., MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, 1985).

The techniques of single chain antibody production such as described, for example, in US Patent NO. 4,946, 778 can equally be used.

Transgenic animals such as mice, for example, can equally be used to produce humanized antibodies.

Agents interacting with the polypeptide of the invention.

The present invention equally has for its object a method for identifying an agent among one or more compounds to be tested which activates or inhibits the activity of a polypeptide according to the invention, said method comprising:

a) providing host cells comprising the recombinant vector according to the invention,

b) contacting said host cells with said compounds to be tested,

c) determining the activating or inhibiting effect upon the activity of said polypeptide whereby said activating or inhibiting agent is identified.

Preferably, the recombinant vector used in said method comprises a polynucleotide according to the invention containing at least one coding SNP.

A polypeptide according to the invention can also be employed for a process for screening compounds that interact with it.

These compounds can be activating (agonists) or inhibiting (antagonists) agents of intrinsic activity of a polypeptide according to the invention. These compounds can equally be ligands or substrates of a polypeptide of the invention. See Coligan et al., Current Protocols in Immunology 1 (2), Chapter 5 (1991).

In general, in order to implement such a process, it is first desirable to produce appropriate host cells that express a polypeptide according to the invention. Such cells can be, for example, cells of mammals, yeasts, insects such as *Drosophila* or bacteria such as *E. coli*.

These cells or membrane extracts of these cells are then put in the presence of the compounds to be tested.

The binding capacity of the compounds to be tested with the polypeptide of the invention can then be observed, as well as the inhibition or the activation of the functional response.

Step c) of the above method can be implemented by using an agent to be tested that is directly or indirectly labeled. It can also include a competition test, by using a labeled or non-labeled agent and a labeled competitor agent.

It can equally be determined if an agent to be tested generates an activation or inhibition signal on cells expressing the polypeptide of the invention by using detection means appropriately chosen according to the signal to be detected.

Such activating or inhibiting agents can be polynucleotides, and in certain cases oligonucleotides or polypeptides, such as proteins or antibodies, for example.

The present invention also has for its object a method for identifying an agent among one or more compounds to be tested whose activity is potentiated or inhibited by a polypeptide according to the invention, said method comprising:

a) providing host cells comprising the recombinant vector according to the invention;

b) contacting said host cells with said compounds to be tested,

c) determining the potentiating or inhibiting effect upon the activity of said agent whereby said potentiated or inhibited agent is identified.

Preferably, the recombinant vector used in said method comprises a polynucleotide according to the invention containing at least one coding SNP.

An agent potentiated or inhibited by the polypeptide of the invention is an agent that responds, respectively, by an activation or an inhibition in the presence of this polypeptide.

The agents, potentiated or inhibited directly or indirectly by the polypeptide of the invention, can consist of polypeptides such as, for example, membranal or nuclear

receptors, kinases and more preferably tyrosine kinases, transcription factor or polynucleotides.

Detection of diseases.

The present invention also has for an object a process for analyzing the biological characteristics of a polynucleotide according to the invention and/or of a polypeptide according to the invention in a subject, comprising at least one of the following:

a) Determining the presence or the absence of a polynucleotide according to the invention in the genome of a subject;

b) Determining the level of expression of a polynucleotide according to the invention in a subject;

c) Determining the presence or the absence of a polypeptide according to the invention in a subject;

d) Determining the concentration of a polypeptide according to the invention in a subject; and/or

e) Determining the functionality of a polypeptide according to the invention in a subject.

These biological characteristics may be analyzed in a subject or in a sample from a subject.

These biological characteristics may permit to carry out a genetic diagnosis and to determine whether a subject is affected or at risk of being affected or, to the contrary, presents a partial resistance to the development of a disease, an indisposition or a disorder linked to the presence of a polynucleotide according to the invention and/or a polypeptide according to the invention.

These diseases can be disorders and/or human diseases, such as:

- disorders or diseases linked to the human growth and development, such as fetal growth and development, perinatal carbohydrate metabolism, phallic growth, craniofacial developments, hypochondroplasia, Laron type of dwarfism,
- disorders related to IGF-1 secretion, such as cognitive functions reduction, mental retardation, sensorineural deafness, insulin resistance, type II diabete,

hematological disorders,

- tumors and cancers such as breast cancer and prostate cancer,
- disorders or diseases linked to the immune system such as allergies, auto-immune diseases, graft rejection, and certain infectious diseases,
- metabolic disorders or diseases related to lipid, nitrogen and/or carbohydrate metabolism such as obesity, arteriosclerosis, body mass maintenance,
- disorders or diseases linked to angiogenesis, retinopathy, cardiovascular diseases.

This process also permits genetic diagnosis of a disease or of a resistance to a disease linked to the presence, in a subject, of the mutant allele encoded by a SNP according to the invention.

Preferably, in step a), the presence or absence of a polynucleotide, containing at least one coding SNP such as previously defined, is going to be detected.

The detection of the polynucleotide may be carried out starting from biological samples from the subject to be studied, such as cells, blood, urine, saliva, or starting from a biopsy or an autopsy of the subject to be studied. The genomic DNA may be used for the detection directly or after a PCR amplification, for example. RNA or cDNA can equally be used in a similar fashion.

It is then possible to compare the nucleotide sequence of a polynucleotide according to the invention with the nucleotide sequence detected in the genome of the subject.

The comparison of the nucleotide sequences can be carried out by sequencing, by DNA hybridization methods, by mobility difference of the DNA fragments on an electrophoresis gel with or without denaturing agents or by melting temperature difference. See Myers et al., Science (1985) 230: 1242. Such modifications in the structure of the nucleotide sequence at a precise point can equally be revealed by nuclease protection tests, such as RNase and the S1 nuclease or also by chemical cleaving agents. See Cotton et al., Proc. Nat. Acad. Sci. USA (1985) 85: 4397-4401. Oligonucleotide probes comprising a polynucleotide fragment of the invention can equally be used to conduct the screening.

Many methods well known to a person skilled in the art can be used to determine the expression of a polynucleotide of the invention and to identify the genetic

variability of this polynucleotide (See Chee et al., Science (1996), Vol 274, pp 610-613).

In step b), the level of expression of the polynucleotide may be measured by quantifying the level of RNA encoded by this polynucleotide (and coding for a polypeptide) according to methods well known to a person skilled in the art as, for example, by PCR, RT-PCR, RNase protection, Northern blot, and other hybridization methods.

In step c) and d) the presence or the absence as well as the concentration of a polypeptide according to the invention in a subject or a sample from a subject may be carried out by well known methods such as, for example, by radioimmunoassay, competitive binding tests, Western blot and ELISA tests.

Consecutively to step d), the determined concentration of the polypeptide according to the invention can be compared with the natural wild-type protein concentration usually found in a subject.

A person skilled in the art can identify the threshold above or below which appears the sensitivity or, to the contrary, the resistance to the disease, the indisposition or the disorder evoked above, with the help of prior art publications or by conventional tests or assays, such as those that are previously mentioned.

In step e), the determination of the functionality of a polypeptide according to the invention may be carried out by methods well known to a person skilled in the art as, for example, by *in vitro* tests such as measuring proliferative activity on rat lymphoma cell line (Nb2, for example) or by an use of host cells expressing said polypeptide.

The present invention also concerns the use of at least one of the following SNPs: g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c, c3693a, in a polynucleotide of the hGH-V gene, for developing diagnostic/prognostic kits for a disease or a resistance to a disease.

The present invention equally concerns a method for diagnosing or determining a prognosis of a disease or a resistance to a disease comprising detecting at least one SNP selected from the group consisting of g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c and c3693a,

in the hGH-V gene.

The invention also concerns a method to identify *in vitro* the presence of at least one SNP selected from the group consisting of g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c and c3693a, in the hGH-V gene of an individual, for determining a possible disease or resistance to a disease caused by the presence of at least one of said SNPs in said individual.

Preferably, the invention concerns a method for diagnosing or determining a prognosis of fetal abnormalities, such as fetal aneuploidy, comprising detecting at least one SNP selected from the group consisting of g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c and c3693a, in the hGH-V gene.

Preferably, the present invention is relative to a method to identify *in vitro* the presence of at least one SNP selected from the group consisting of g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c and c3693a, in the hGH-V gene of an individual, for determining possible fetal abnormalities, such as fetal aneuploidy, caused by the presence of at least one of said SNPs in said individual.

The detection of at least one SNP of the invention may be carried out starting from biological samples from the subject to be studied, such as cells, blood, urine, saliva, or starting from a biopsy or an autopsy of the subject to be studied. The genomic DNA may be used for the detection directly or after a PCR amplification, for example. RNA or cDNA can equally be used in a similar fashion.

It is then possible to compare the nucleotide sequence of the polynucleotide isolated from the subject with the nucleotide sequence of a polynucleotide comprising at least one SNP of the invention.

The comparison of the nucleotide sequences can be carried out by sequencing, by DNA hybridization methods, by mobility difference of the DNA fragments on an electrophoresis gel with or without denaturing agents or by melting temperature difference. See Myers et al., Science (1985) 230: 1242. Such modifications in the structure of the nucleotide sequence at a precise point can equally be revealed by nuclease protection tests, such as RNase and the S1 nuclease or also by chemical

cleaving agents. See Cotton et al., Proc. Nat. Acad. Sci. USA (1985) 85: 4397-4401. A combination of DNA hybridization methods and enzymatic digestion may also be used as in the Invader assay. Oligonucleotide probes comprising a polynucleotide fragment of the invention can equally be used to conduct the screening.

The present invention has also as an object a diagnostic kit comprising one or more of: an isolated polynucleotide according to the invention ; a previously defined recombinant vector ; a previously defined host cell ; a polypeptide according to the invention ; a previously defined antibody.

Therapeutic agents and treatments of diseases.

The present invention also has for its object a therapeutic agent containing, by way of active agent, a polypeptide according to the invention.

The invention also relates to the use of a therapeutically effective amount of a polypeptide according to the invention for the manufacture of a medicament for preventing or treating in an individual a disease or a disorder from the group consisting of:

- disorders or diseases linked to the human growth and development, such as fetal growth and development, perinatal carbohydrate metabolism, phallic growth, craniofacial developments, hypochondroplasia, Laron type of dwarfism,
- disorders related to IGF-1 secretion, such as cognitive functions reduction, mental retardation, sensorineural deafness, insulin resistance, type II diabete, hematological disorders,
- tumors and cancers such as breast cancer and prostate cancer,
- disorders or diseases linked to the immune system such as allergies, auto-immune diseases, graft rejection, and certain infectious diseases,
- metabolic disorders or diseases related to lipid, nitrogen and/or carbohydrate metabolism such as obesity, arteriosclerosis, body mass maintenance,
- disorders or diseases linked to angiogenesis, retinopathy, cardiovascular diseases.

Certain of the compounds permitting to obtain the polypeptide according to the invention as well as the compounds obtained or identified by or from this polypeptide can likewise be used for the therapeutic treatment of the human body, i.e. as a therapeutic agent.

This is why the present invention also has for an object a medicament containing, by way of active agent, a polynucleotide according to the invention containing at least one coding SNP, a previously defined recombinant vector, a previously defined host cell, and/or a previously defined antibody.

The present invention also concerns a therapeutic agent comprising one or more compounds selected from the group consisting of an isolated polynucleotide according to the invention ; a previously defined recombinant vector ; a previously defined host cell ; a previously defined polypeptide ; a previously defined antibody.

Preferably, the therapeutic agent described above contains a polynucleotide according to the invention containing at least one coding SNP.

The invention also relates to the use of a therapeutically effective amount of a therapeutic agent comprising a polynucleotide according to the invention, a previously defined recombinant vector, a previously defined host cell, and/or a previously defined antibody, for the preparation of a medicament for preventing or treating in an individual a disease or disorder selected from the group consisting of:

- disorders or diseases linked to the human growth and development, such as fetal growth and development, perinatal carbohydrate metabolism, phallic growth, craniofacial developments, hypochondroplasia, Laron type of dwarfism,
- disorders related to IGF-1 secretion, such as cognitive functions reduction, mental retardation, sensorineural deafness, insulin resistance, type II diabete, hematological disorders,
- tumors and cancers such as breast cancer and prostate cancer,
- disorders or diseases linked to the immune system such as allergies, auto-immune diseases, graft rejection, and certain infectious diseases,
- metabolic disorders or diseases related to lipid, nitrogen and/or carbohydrate metabolism such as obesity, arteriosclerosis, body mass maintenance,
- disorders or diseases linked to angiogenesis, retinopathy, cardiovascular diseases.

Preferably, the polynucleotide used as therapeutic agent as described above contains at least one coding SNP.

The dosage of a polypeptide and of the other compounds of the invention, useful as active agent, depends on the choice of the compound, the therapeutic indication, the

mode of administration, the nature of the formulation, the nature of the subject and the judgment of the doctor.

When it is used as active agent, a polypeptide according to the invention is generally administered at doses ranging between 0.05 and 0.5 mg/kg of the subject, per week and according to the mode of administration.

The invention also has as an object a pharmaceutical composition that contains, as active agent, at least one above-mentioned compound such as a polypeptide according to the invention, a polynucleotide according to the invention containing at least one previously defined SNP, a previously defined recombinant vector, a previously defined host cell, and/or a previously defined antibody, as well as a pharmaceutically acceptable excipient.

In these pharmaceutical compositions, the active agent is advantageously present at physiologically effective doses.

These pharmaceutical compositions can be, for example, solids or liquids and be present in pharmaceutical forms currently used in human medicine such as, for example, simple or coated tablets, gelcaps, granules, caramels, suppositories and preferably injectable preparations and powders for injectables. These pharmaceutical forms can be prepared according to usual methods.

The active agent(s) can be incorporated into excipients usually employed in pharmaceutical compositions such as talc, Arabic gum, lactose, starch, dextrose, glycerol, ethanol, magnesium stearate, cocoa butter, aqueous or non-aqueous vehicles, fatty substances of animal or vegetable origin, paraffinic derivatives, glycols, various wetting agents, dispersants or emulsifiers, preservatives.

The active agent(s) according to the invention can be employed alone or in combination with other compounds such as therapeutic agents such as adult human growth hormone, interferons, even other cytokines such as interleukins, for example.

The different formulations of the pharmaceutical compositions are adapted according to the mode of administration.

The pharmaceutical compositions can be administered by different routes of administration known to a person skilled in the art.

The invention equally has for an object a diagnostic composition that contains, as active agent, at least one above-mentioned compound such as a polypeptide according to the invention, all or part of a polynucleotide according to the invention, a previously defined recombinant vector, a previously defined host cell, and/or a previously defined antibody, as well as a suitable pharmaceutically acceptable excipient.

This diagnostic composition may contain, for example, an appropriate excipient like those generally used in the diagnostic composition such as buffers and preservatives.

The present invention equally has as an object the use:

a) of a therapeutically effective quantity of a polypeptide according to the invention, and/or

b) of a polynucleotide according to the invention, and/or

c) of a host cell from the subject to be treated, previously defined,

to prepare a therapeutic agent intended to increase the expression or the activity, in a subject, of a polypeptide according to the invention.

Thus, to treat a subject who needs an increase in the expression or in the activity of a polypeptide of the invention, several methods are possible.

It is possible to administer to the subject a therapeutically effective quantity of a polypeptide of the invention, with a pharmaceutically acceptable excipient.

It is likewise possible to increase the endogenous production of a polypeptide of the invention by administration to the subject of a polynucleotide according to the invention. For example, this polynucleotide can be inserted in a retroviral expression vector. Such a vector can be isolated starting from cells having been infected by a retroviral plasmid vector containing RNA encoding for the polypeptide of the invention, in such a fashion that the transduced cells produce infectious viral particles containing the gene of interest. See Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, Chapter 20, in Human Molecular Genetics, Strachan and Read, BIOS Scientifics Publishers Ltd (1996).

In accordance with the invention, a polynucleotide containing at least one coding SNP such as previously defined will be preferably used.

It is equally possible to administer to the subject host cells belonging to him,

these host cells having been preliminarily taken and modified so as to express the polypeptide of the invention, as previously described.

The present invention equally relates to the use:

a) of a therapeutically effective quantity of a previously defined immunospecific antibody, and/or

b) of a polynucleotide permitting inhibition of the expression of a polynucleotide according to the invention,

in order to prepare a therapeutic agent intended to reduce the expression or the activity, in a subject, of a polypeptide according to the invention.

Thus, it is possible to administer to the subject a therapeutically effective quantity of an inhibiting agent and/or of an antibody such as previously defined, possibly in combination, with a pharmaceutically acceptable excipient.

It is equally possible to reduce the endogenous production of a polypeptide of the invention by administration to the subject of a complementary polynucleotide according to the invention permitting inhibition of the expression of a polynucleotide of the invention.

Preferably, a complementary polynucleotide containing at least one coding SNP such as previously defined can be used.

The present invention concerns the use of a therapeutically effective quantity of one or more of: an isolated polynucleotide according to the invention ; a previously defined recombinant vector ; a previously defined host cell ; a polypeptide according to the invention; a previously defined antibody,

for the preparation of a medicament for increasing or decreasing the activity in a subject of a polypeptide according to the invention.

The present invention equally concerns the use of a therapeutically effective amount of one or more of: an isolated polynucleotide according to the invention ; a previously defined recombinant vector ; a previously defined host cell ; a polypeptide according to the invention; a previously defined antibody,

for the preparation of a medicament for preventing or treating in an individual a disorder or a disease linked to the presence in the genome of said individual of a polynucleotide according to the invention.

The present invention concerns also the use of a growth hormone for the preparation of a medicament for the prevention or the treatment of an individual having a disorder or a disease caused by a hGH-V variant linked to the presence in the genome of said individual of a nucleotide sequence having at least 95% identity (preferably, at least 97% identity, more preferably at least 99% identity and particularly 100% identity) with the nucleotide sequence SEQ ID NO. 1, provided that said nucleotide sequence comprises one of the following SNPs: g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c, c3693a.

The growth hormone is preferably the placental growth hormone (PGH) and/or the adult growth hormone.

The growth hormone is particularly useful for the prevention or the treatment of disorders or diseases selected among:

- disorders or diseases linked to the human growth and development, such as fetal growth and development, perinatal carbohydrate metabolism, phallic growth, craniofacial developments, hypochondroplasia, Laron type of dwarfism,
- disorders related to IGF-1 secretion, such as cognitive functions reduction, mental retardation, sensorineural deafness, insulin resistance, type II diabete, hematological disorders,
- tumors and cancers such as breast cancer and prostate cancer,
- disorders or diseases linked to the immune system such as allergies, auto-immune diseases, graft rejection, and certain infectious diseases,
- metabolic disorders or diseases related to lipid, nitrogen and/or carbohydrate metabolism such as obesity, arteriosclerosis, body mass maintenance,
- disorders or diseases linked to angiogenesis, retinopathy, cardiovascular diseases.

The individual may be an adult, an adolescent, a child or a fetus.

Preferably, the individual is a fetus or a child less than twelve years old such as for example an infant just after birth.

CLAIMS

1. An isolated polynucleotide comprising:

a) All or part of the nucleotide sequence SEQ ID NO. 1, provided that such nucleotide sequence, or part of sequence, comprises at least one SNP selected from the group consisting of g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c and c3693a; or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

2. An isolated polynucleotide comprising:

a) All or part of the nucleotide sequence SEQ ID NO. 2, provided that said sequence, or part of sequence, contains at least one coding SNP selected from the group consisting of c51a and t454g; or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

3. An isolated polynucleotide comprising:

a) All or part of the nucleotide sequence SEQ ID NO. 3, provided that said sequence, or part of sequence, contains at least one coding SNP selected from the group consisting of c51a, t454g, g628a and t721c; or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

4. An isolated polynucleotide comprising:

a) All or part of the nucleotide sequence SEQ ID NO. 4, provided that said sequence, or part of sequence, contains at least one coding SNP selected from the group consisting of c51a, t464c, a683c and c721a; or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

5. An isolated polynucleotide comprising:

a) All or part of the nucleotide sequence SEQ ID NO. 5, provided that said sequence, or part of sequence, contains at least one coding SNP selected from the group consisting of c51a and t409g; or

b) A nucleotide sequence complementary to a nucleotide sequence under a).

6. The isolated polynucleotide according to any one of claims 1 to 5, wherein said polynucleotide is composed of at least 10 nucleotides.

7. An isolated polynucleotide consisting of:

a) The nucleotide sequence SEQ ID NO. 6, or

b) A nucleotide sequence comprising the nucleotide sequence under a) and at most 100 additional nucleotides, and preferably at most 50 additional nucleotides, at the

5' end, or at the 3' end, or at each of the 5' and 3' ends of said nucleotide sequence under a) ; or

c) A nucleotide sequence complementary to one of the nucleotide sequences under a) or b).

8. An isolated polynucleotide that codes for a chimeric polypeptide comprising the amino acid sequence SEQ ID NO. 7.

9. An isolated polynucleotide that codes for a polypeptide consisting of the amino acid sequence SEQ ID NO. 7.

10. An isolated polynucleotide that codes for a polypeptide comprising all or part of the amino acid sequence SEQ ID NO. 10, provided that said sequence, or part of sequence, contains the coding SNP W152G.

11. An isolated polynucleotide that codes for a polypeptide comprising all or part of the amino acid sequence SEQ ID NO. 11, provided that said sequence, or part of sequence, contains at least one coding SNP selected from the group consisting of W152G, E210K and W241R.

12. An isolated polynucleotide that codes for a polypeptide comprising all or part of the amino acid sequence SEQ ID NO. 12, provided that said sequence, or part of sequence, contains at least one coding SNP selected from the group consisting of M155T, Q228P and P241T.

13. An isolated polynucleotide that codes for a polypeptide comprising all or part of the amino acid sequence SEQ ID NO. 13, provided that said sequence, or part of sequence, contains the coding SNP W137G.

14. A method for identifying or amplifying all or part of a polynucleotide having 90 to 100% identity with nucleotide sequence SEQ ID NO. 1, comprising hybridizing under appropriate hybridization conditions said polynucleotide with a polynucleotide according to any one of claims 1 to 13.

15. A method for genotyping all or part of a polynucleotide having 90 to 100% identity with nucleotide sequence SEQ ID NO. 1, comprising the steps of amplifying a region of interest in the genomic DNA of a subject or a population of subjects, and determining the allele of at least one position in the nucleotide sequence SEQ ID NO. 1 chosen from the group consisting of: 2139, 2197, 2204, 2205, 2206, 2267, 2435, 2465, 2635, 3169, 3267, 3343, 3436, 3655 and 3693.

16. The method of claim 15, wherein the genotyping is carried out by minisequencing.

17. A recombinant vector comprising a polynucleotide according to any one of claims 1 to 13.

18. A host cell comprising a recombinant vector according to claim 17.

19. A method for separating a polypeptide, comprising cultivating a host cell according to claim 18 in a culture medium and separating said polypeptide from the culture medium.

20. The polypeptide encoded by the isolated polynucleotide of claim 1.

21. An isolated polypeptide consisting of the amino acid sequence SEQ ID NO. 7.

22. A chimeric polypeptide comprising the amino acid sequence SEQ ID NO. 7.

23. An isolated polypeptide comprising all or part of the amino acid sequence SEQ ID NO. 10, provided that said sequence, or part of sequence, contains the coding SNP W152G.

24. An isolated polypeptide comprising all or part of the amino acid sequence SEQ ID NO. 11, provided that said sequence, or part of sequence, contains at least one coding SNP selected from the group consisting of W152G, E210K and W241R.

25. An isolated polypeptide comprising all or part of the amino acid sequence SEQ ID NO. 12, provided that said sequence, or part of sequence, contains at least one coding SNP selected from the group consisting of M155T, Q228P and P241T.

26. An isolated polypeptide comprising all or part of the amino acid sequence SEQ ID NO. 13, provided that said sequence, or part of sequence, contains the coding SNP W137G.

27. An isolated polypeptide comprising amino acids 27 through 217 of amino acid sequence SEQ ID NO. 10 and having SNP W152G.

28. An isolated polypeptide comprising amino acids 27 through 256 of amino acid sequence SEQ ID NO. 11 and having at least one coding SNP selected from the group consisting of W152G, E210K and W241R.

29. An isolated polypeptide comprising amino acids 27 through 245 of amino acid sequence SEQ ID NO. 12 and having at least one coding SNP selected from the group consisting of M155T, Q228P and P241T.

30. An isolated polypeptide comprising amino acids 27 through 202 of amino acid sequence SEQ ID NO. 13 and having SNP W137G.

31. An isolated polypeptide which is encoded by all or part of the nucleotide sequence SEQ ID NO. 1, provided that said sequence or said part thereof comprises the SNP t3169g.

32. An isolated polypeptide which is encoded by a nucleotide sequence selected in the group consisting of:

a) a nucleotide sequence SEQ ID NO. 2; or

b) a part of nucleotide sequence SEQ ID NO. 2; or

c) a nucleotide sequence SEQ ID NO. 3; or

d) a part of nucleotide sequence SEQ ID NO. 3,

provided that the sequences under a), b), c) or d) comprise the SNP t454g.

33. An isolated polypeptide which is encoded by all or part of the nucleotide sequence SEQ ID NO. 5, provided that said sequence or said part thereof comprises the SNP t409g.

34. A method for obtaining an immunospecific antibody, comprising immunizing an animal with a polypeptide according to any one of claims 20 to 33, and collecting said antibody from said animal.

35. The immunospecific antibody resulting from the method of claim 34.

36. A method for identifying an agent among one or more compounds to be tested which activates or inhibits the activity of a polypeptide according to any one of claims 20 to 33, said method comprising:

a) providing host cells comprising the recombinant vector according to claim 17;

b) contacting said host cells with said compounds to be tested,

c) determining the activating or inhibiting effect upon the activity of said polypeptide whereby said activating or inhibiting agent is identified.

37. A method for identifying an agent among one or more compounds to be tested whose activity is potentiated or inhibited by a polypeptide according to any one of claims 20 to 33, said method comprising:

a) providing host cells comprising the recombinant vector according to claim 17;

b) contacting said host cells with said compounds to be tested,

c) determining the potentiating or inhibiting effect upon the activity of said agent whereby said potentiated or inhibited agent is identified.

38. The method according to any one of claims 36 and 37, wherein said recombinant vector comprises a polynucleotide according to any one of claims 1 to 13 and having at least one coding SNP.

39. A method for analyzing the biological characteristics of a subject, comprising performing at least one of the following steps:

a) Determining the presence or the absence of a polynucleotide according to any one

of claims 1 to 13 in the genome of a subject;

b) Determining the level of expression of a polynucleotide according to any one of claims 1 to 13 in a subject;

c) Determining the presence or the absence of a polypeptide according to any one of claims 20 to 33 in a subject;

d) Determining the concentration of a polypeptide according to any one of claims 20 to 33 in a subject; or

e) Determining the functionality of a polypeptide according to any one of claims 20 to 33 in a subject.

40. A therapeutic agent comprising one or more compounds selected from the group consisting of an isolated polynucleotide according to any one of claims 1 to 13 ; a recombinant vector according to claim 17 ; a host cell according to claim 18 ; a polypeptide according to any one of claims 20 to 33 ; an antibody according to claim 35.

41. Use of a therapeutically effective amount of a therapeutic agent according to claim 40 for the preparation of a medicament for preventing or treating in an individual a disease or a disorder selected from the group consisting of:

- disorders or diseases linked to the human growth and development, such as fetal growth and development, perinatal carbohydrate metabolism, phallic growth, craniofacial developments, hypochondroplasia, Laron type of dwarfism,
- disorders related to IGF-1 secretion, such as cognitive functions reduction, mental retardation, sensorineural deafness, insulin resistance, type II diabete, hematological disorders,
- tumors and cancers such as breast cancer and prostate cancer,
- disorders or diseases linked to the immune system such as allergies, auto-immune diseases, graft rejection, and certain infectious diseases,
- metabolic disorders or diseases related to lipid, nitrogen and/or carbohydrate metabolism such as obesity, arteriosclerosis, body mass maintenance,
- disorders or diseases linked to angiogenesis, retinopathy, cardiovascular diseases.

42. Use of a therapeutically effective quantity of one or more of:

an isolated polynucleotide according to any one of claims 1 to 13 ; a recombinant vector according to claim 17 ; a host cell according to claim 18 ; a polypeptide according to any one of claims 20 to 33 ; an antibody according to claim 35,

for the preparation of a medicament for increasing or decreasing the activity in a subject of a polypeptide according to any one of claims 20 to 33.

43. Use of a therapeutically effective amount of one or more of:
an isolated polynucleotide according to any one of claims 1 to 13 ; a recombinant vector according to claim 17 ; a host cell according to claim 18 ; a polypeptide according to any one of claims 20 to 33 ; an antibody according to claim 35,
for the preparation of a medicament for preventing or treating in an individual a disorder or a disease linked to the presence in the genome of said individual of a polynucleotide according to any one of claims 1 to 13.

44. The use of claim 43 wherein the individual is a fetus or a child.

45. A method for determining statistically relevant associations between at least one SNP selected from the group consisting of g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c and c3693a, in the hGH-V gene, and a disease or resistance to disease comprising :
a) Genotyping a group of individuals;
b) Determining the distribution of said disease or resistance to disease within said group of individuals;
c) Comparing the genotype data with the distribution of said disease or resistance to disease; and
d) Analyzing said comparison for statistically relevant associations.

46. A method for diagnosing or determining a prognosis of a disease or a resistance to a disease comprising detecting at least one SNP selected from the group consisting of g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c and c3693a, in the hGH-V gene.

47. A method to identify *in vitro* the presence of at least one SNP selected from the group consisting of g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c and c3693a, in the hGH-V gene of an individual, for determining a possible disease or resistance to a disease caused by the presence of at least one of said SNPs in said individual.

48. A method for diagnosing or determining a prognosis of fetal abnormalities, such as fetal aneuploidy, comprising detecting at least one SNP selected from the group consisting of g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c and c3693a, in the hGH-V gene.

49. A method to identify *in vitro* the presence of at least one SNP selected from the group consisting of g2139c, g2197t, g2204a, g2205t, c2206t, g2267a, g2435a, c2465a, a2635c, t3169g, g3267c, g3343a, t3436c, a3655c and c3693a, in the hGH-V gene of an individual, for determining possible fetal abnormalities, such as fetal aneuploidy, caused by the presence of at least one of said SNPs in said individual.

50. Diagnostic kit comprising one or more of:
an isolated polynucleotide according to any one of claims 1 to 13 ; a recombinant vector according to claim 17 ; a host cell according to claim 18 ; a polypeptide according to any one of claims 20 to 33 ; an antibody according to claim 35.

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(10) International Publication Number
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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
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4 September 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/101002 A3

(54) Title: IDENTIFICATION OF SNPS THE HGV-V GENE

(57) Abstract: The present invention relates to new polynucleotides deriving from the nucleotide sequence of the hGH-V gene and comprising SNP(s), new polypeptides comprising a mutation caused by this (these) SNP(s) as well as their therapeutic uses.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/08919

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/00 C12N15/85 C07K14/61 C12Q1/68 A61K38/27
A61K39/395 A61K48/00 G01N33/50 C07K16/26 C12N5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, MEDLINE, EMBASE, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE ENTREZ SNP 'Online! 13 September 2000 (2000-09-13) retrieved from NCBI Database accession no. rs1130670 XP002234490 abstract ---	1-50
X	DATABASE ENTREZ SNP 'Online! 13 September 2000 (2000-09-13) retrieved from NCBI Database accession no. rs1130686 XP002234491 abstract --- -/--	1-50

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 March 2003	08/04/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Petri, B

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE ENTREZ SNP 'Online! 13 September 2000 (2000-09-13) retrieved from NCBI Database accession no. rs1058053 XP002234492 abstract	1-50
X	DATABASE ENTREZ SNP 'Online! 13 September 2000 (2000-09-13) retrieved from NCBI Database accession no. rs1058051 XP002234493 abstract	1-50
X	DATABASE ENTREZ SNP 'Online! 13 September 2000 (2000-09-13) retrieved from NCBI Database accession no. rs1050724 XP002234494 abstract	1-50
X	DATABASE ENTREZ SNP 'Online! 13 September 2000 (2000-09-13) retrieved from NCBI Database accession no. rs1050840 XP002234495 abstract	1-50
X	DATABASE ENTREZ SNP 'Online! 13 September 2000 (2000-09-13) retrieved from NCBI Database accession no. rs1048692 XP002234496 abstract	1-50
X	WO 95 20398 A (HUMAN GENOME SCIENCES INC) 3 August 1995 (1995-08-03) the whole document page 34 -page 36; claims 21,22	8
A	DATABASE OMIM 'Online! 18 March 1999 (1999-03-18) "GROWTH HORMONE 2; GH2" retrieved from NCBI Database accession no. 139240 XP002234497 abstract	1-50

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	NICKERSON DEBORAH A ET AL: "PolyPhred: Automating the detection and genotyping of single nucleotide substitutions using fluorescence-based resequencing" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 25, no. 14, 1997, pages 2745-2751, XP002152564 ISSN: 0305-1048 the whole document	1-50
A	BARTLETT SYLVIA ET AL: "Alkaline-mediated differential interaction (AMDI): A simple automatable single-nucleotide polymorphism assay." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 98, no. 5, 27 February 2001 (2001-02-27), pages 2694-2697, XP002185148 February 27, 2001 ISSN: 0027-8424 the whole document	1-50
A	LEHNERT VALERIE ET AL: "A semi-automated system for analysis and storage of SNPs." HUMAN MUTATION, vol. 17, no. 4, 2001, pages 243-254, XP002185149 ISSN: 1059-7794 abstract	1-50

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-6, 10-13, 23-26, 32-33 (all partially)

Said claims define polynucleotides/polypeptides comprising parts of undefined length of particular reference molecules. Said definitions are technically meaningless as their scope is indefinite. This applies also to claim 6 as the specified sequence length of 10 nts refers back to the molecule comprising parts of undefined length.
The search has been restricted to sequences comprising all of the specified Seq. Id. Nos.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 02/08919

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-6, 10-13, 23-26, 32-33 (all partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna[illegible]al Application No
PCT/EP 02/08919

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9520398 A	03-08-1995	US 5597709 A	28-01-1997
		AU 681260 B2	21-08-1997
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		CA 2182205 A1	03-08-1995
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		NZ 281761 A	24-11-1997
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		US 5962411 A	05-10-1999